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# Health Assessment Document for Vinylidene Chloride Final Report

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16. ABSTRACT

Vinylidene chloride is a highly reactive, flammable, clear colorless liquid. In the absence of chemical inhibitors, it can produce violently explosive, complex peroxides. The estimated, ambient air level of vinylidene chloride in urban-suburban areas of the United States is 20ng/m$^3$. The median concentration (14 ug/m$^3$) for ambient air at point sources of emission is substantially higher.

Following oral or inhalation exposure, mammals readily absorb vinylidene chloride. Metabolites of vinylidene chloride, formed in the liver, produce toxic lesions in the liver and kidneys (the target organs), with inhibitors of metabolism providing protection from vinylidene chloride toxicity. The acute hepatotoxicity of vinylidene chloride is greater than that of any other chloroethylene.

Based on the overall evidence, VDC would be a Group 3 chemical according to the IARC (International Agency for Research in Cancer's) approach, and by definition, a Group 3 chemical cannot be classified according to its carcinogenicity to humans. However, the mutagenic activity of VDC, its chemical structure, its activity as a tumor initiator in mouse skin, and the ability of its metabolites to react with DNA raise concern that the total body of evidence should not be lightly dismissed.

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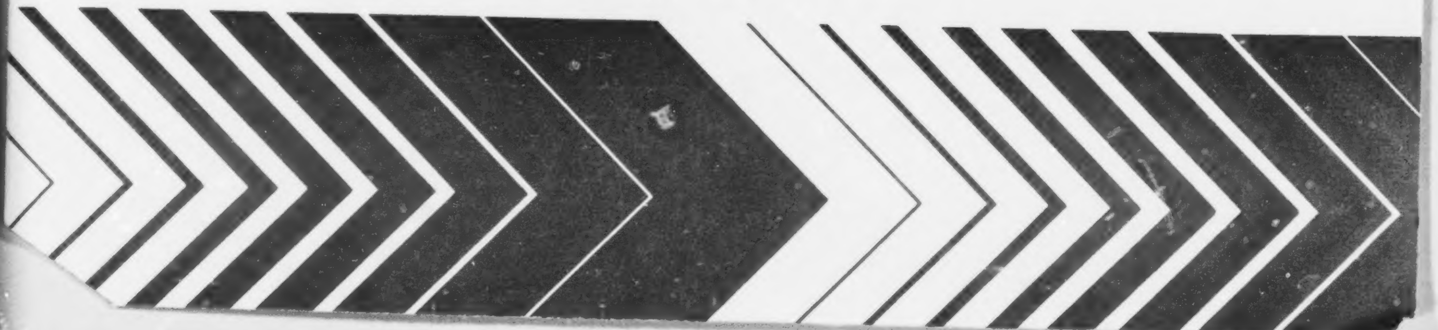

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# Health Assessment Document for Vinylidene Chloride

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
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# DISCLAIMER

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# PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for EPA use. The health assessment document was originally developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of vinylidene chloride as a hazardous air pollutant. However, the scope of this document has since been expanded to address multimedia aspects.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

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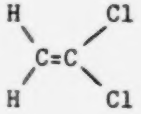
# 1. SUMMARY AND CONCLUSIONS

Vinylidene chloride is a highly reactive, flammable, clear, colorless liquid that, in the presence of air, can form complex peroxides in the absence of chemical inhibitors. The peroxides are violently explosive, and formaldehyde, phosgene, and hydrochloric acid are produced as decomposition products. Vinylidene chloride has a boiling point of 31.6°C at 760 mm Hg and a vapor pressure of 600 mm Hg at 25°C. The solubility of vinylidene chloride in water is 2250 mg/l at 25°C, and the density of the liquid is 1.2132 g/cm$^3$ (20°C). Vinylidene chloride vapor is 3.34 times as dense as air.

Synonyms for vinylidene chloride are 1,1-dichloroethene, 1,1-DCE, and 1,1-dichloroethylene. Vinylidene chloride has a molecular weight of 96.95 and a molecular formula of $C_2H_2Cl_2$. The structural formula is given below.



Vinylidene chloride monomer production capacity in the United States is approximately 178 million pounds per year. Virtually all of the vinylidene chloride produced is used in the production of copolymers with vinyl chloride or acrylonitrile. A small percentage (4%) of vinylidene chloride is used as chemical intermediates.

The two primary methods that have been used in recent years for the sampling and analysis of vinylidene chloride in ambient air are the freeze-trap method and the sorption onto Tenax-GC method with subsequent analysis of the desorbed vinylidene chloride by high resolution gas chromatography with either flame ionization, electron capture, electrical conductivity, or mass spectrometric detectors. Both the freeze-trap and Tenax-GC methods of sample collection have some disadvantages. For example, the freeze-trap method using liquid oxygen is a cumbersome method both for sample collection and transportation, and the Tenax-GC method may suffer from serious problems of artifact formation.

The two methods commonly used for the analysis of vinylidene chloride in grab aqueous samples are the static head-space method and the dynamic purge-trap method. However, for aqueous samples containing very low levels of vinylidene chloride (e.g., potable water), the dynamic purge-trap method is more suitable than the static head-space method because of the higher sensitivity of the former. Although gas chromatography with either flame ionization, electron capture, electrical conductivity or mass spectrometric detectors has been used for the final quantification of vinylidene chloride by both methods, the electrical conductivity detector is preferable to other detectors because of its greater sensitivity and selectivity. The mass spectrometric method is usually used as a confirmatory technique.

Vinylidene chloride in soil samples has been analyzed by solvent extraction in sealed vials with subsequent quantification by gas chromatography - flame ionization detector (GC-FID), and using mass spectrometry as the confirmatory technique. The analysis of vinylidene chloride in food wrapping materials, foods, and biological tissues has been performed either by the static head-space method or by the dynamic purge-trap method in a manner similar to that employed for aqueous samples.

Due to its high volatility, vinylidene chloride is lost to the atmosphere during industrial manufacture of the monomer and polymer, and during storage and handling. The total emission of vinylidene chloride to all media from these facilities has been estimated to be 1,300,400 pounds per year. Moreover, vinylidene chloride originally in aqueous solution is likely to contribute to air contamination as a result of its high volatility from water.

Under atmospheric smog conditions, the half-life of vinylidene chloride in air has been determined to be 5 to 12 hours. In the absence of smog conditions, vinylidene chloride may persist in the atmosphere with a half-life of approximately 2 days. Volatilization from aquatic media is probably the most significant fate-determining process for vinylidene chloride, although the role of biodegradation still remains uncertain. The half-lives of volatilization of vinylidene chloride from pond, river, and lake water have been estimated to be 6.1 days, 1.2 days, and 4.2 days, respectively. The fate of vinylidene chloride in soils has not been evaluated with certainty. However, it has been concluded from the limited data that both volatilization and leaching may play significant roles in determining the fate of this chemical in soils.

The median ambient air level of vinylidene chloride in urban/suburban areas of the U.S. was estimated to be 5 ppt (20 ng/$m^3$). However, the median concentration value is substantially higher 2182 ppt (8.66 μg/$m^3$) for ambient air in the vicinity of point sources of emission. The estimated daily vinylidene chloride intake from ambient air in urban/suburban areas through inhalation is 0.4 μg. However, the daily inhalation exposure from ambient air may be as high as 0.17 mg in the immediate vicinity of point sources. Vinylidene chloride has been detected in approximately 3% of the total drinking water supplies in the U.S. at an estimated mean concentration of 0.3 μg/l and a concentration range of 0.2 to 0.5 μg/l. For the majority of the U.S. population, the daily exposure to vinylidene chloride from ingestion of drinking water has been estimated to be less than 0.6 μg, although the maximum daily exposure in certain communities could exceed 1 μg. Because of the paucity of data, no estimate of the dietary intake of vinylidene chloride in the U.S. can be made at the present time.

Vinylidene chloride is acutely toxic to aquatic animals at exposure concentrations in the milligrams per liter range. The lowest concentration reported to be acutely toxic to an aquatic organism is 2.4 mg/l. Reported acute and subchronic $LC_{50}$ values ranged between 11.6 and 250 mg/l for aquatic animals. Vinylidene chloride was not acutely toxic to aquatic algae at concentrations of 712 to 798 mg/l. Vinylidene chloride was, however, toxic to yeast. No information was found concerning the toxicity of vinylidene chloride to domestic animals and non-aquatic wildlife.

Vinylidene chloride is readily absorbed by mammals following oral or inhalation exposure. Vinylidene chloride is metabolized in the liver with a number of possible reactive intermediates, including an epoxide, being formed. These reactive intermediate metabolites can react with macromolecules; this is a characteristic of many chemical carcinogens. The metabolites of vinylidene chloride produce toxic lesions in the liver and kidneys, with inhibitors of metabolism providing protection from vinylidene chloride toxicity. The hepatotoxic effect can be extensive and histological effects can be noted within 2 hours after the onset of exposure. The acute hepatotoxicity of vinylidene chloride was shown to be greater than that of any other chloroethylene. The liver and kidneys remain the target organs for toxic effects regardless of the route of administration (administration vehicle may influence vinylidene chloride metabolism and therefore affect the extent of toxicity) or whether acute, subacute, or chronic exposure occurs.

Vinylidene chloride has been described as a possible weak teratogen in a study using rats and mice. In this study, the experimental levels of vinylidene chloride were toxic to the dams, confounding the interpretation of the results. It was clear, however, that exposure of pregnant rats and mice to high levels of vinylidene chloride could cause fetotoxicity and adversely affect the outcome of pregnancy. Another study reported no teratogenic effect in rats or rabbits inhaling up to 160 ppm vinylidene chloride for 7 hours per day or in rats given drinking water containing 200 ppm vinylidene chloride. Embryo and maternal toxicity were observed among rats inhaling 80 or 160 ppm vinylidene chloride and rabbits inhaling 160 ppm. At exposures causing little or no maternal toxicity (20 ppm in rats, 80 ppm in rabbits), there was no effect on embryo or fetal development. The effect of vinylidene chloride on reproduction and fetal and neonatal development of rats was tested in a three generation study with six litter groups being produced. Hepatocellular fatty changes were observed in rats ingesting 50, 100 or 200 ppm vinylidene chloride.

A large number of studies indicate that vinylidene chloride is mutagenic to bacteria and that this activity is largely dependent on microsomal activation. Vinylidene chloride was reported to produce positive results for gene reversion and conversion in yeast, which was also dependent on metabolic activation, and was positive in Tradescantia. In mammalian systems, vinylidene chloride failed to induce gene mutations in V79 cells at two separate loci, failed to induce cromosomal aberrations in mouse bone marrow in vivo, and failed to induce dominant lethals in either mice or rats. Vinylidene chloride was found to alkylate the DNA of mice exposed through inhalation and may have caused unscheduled DNA synthesis in the kidneys of similarly exposed mice.

Analysis of the data relating to the potential of vinylidene chloride to behave as a human germ-cell mutagen indicates that based on the criteria established in the Agency's Proposed Guidelines for Mutagenicity Risk Assessment, the evidence at the present time is classified as limited. This designation indicates that there are insufficient data on either mutagenicity or interaction with germ cells to classify the evidence as either sufficient or suggestive of potential germ-cell mutagenicity. However, available data also do not permit the classification of vinylidene chloride as a non-germ-cell mutagen.

Based on the limited evidence from animal studies, supporting evidence from mutagenicity studies, and related biochemical and toxicity considerations, it is recommended that vinylidene chloride be considered a "possible" carcinogen for humans.

A total of 18 chronic studies in animals were evaluated for evidence of carcinogenicity. The exposure regimes for these studies were as follows: 11 were inhalation, 4 were gavage, 1 was drinking water, 1 was subcutaneous injection, and 1 was skin application. Evidence for carcinogenicity was found in Swiss mice exposed to vinylidene chloride by inhalation, 4 hours daily for 12 months. A statistically significant increase of kidney adenocarcinomas, a rare tumor type, was observed in male mice. Statistically significant increases in mammary carcinomas and pulmonary adenomas were observed in mice of both sexes, although the importance of these findings is uncertain because no clear dose-response relationship was evident. Vinylidene chloride has also been shown to be a tumor initiator in mouse skin. The remaining 16 animal studies have negative (assuming non-treatment related increases are also negative) findings for carcinogenicity; however, these negative findings may be partially explained by study characteristics such as, less than lifetime dosing, below maximum tolerated dose levels, and single dose studies, which individually or in combination, reduce the sensitivity of detecting a carcinogenic response. While the number of studies are many, the inadequacy of test conditions demonstrates the need for additional testing to elucidate the potential for human carcinogenicity. The mutagenic activity of vinylidene chloride, its chemical structure, its activity as a tumor initiator in mouse skin, and the ability of metabolites to react with DNA, signal that the animal evidence should not be lightly dismissed.

There is only one epidemiologic study for vinylidene chloride. While the study concluded that a carcinogenic effect could not be attributed to vinylidene chloride, limiting characteristics made it inadequate to evaluate the carcinogenic potential.

The carcinogenicity evidence has been evaluated using the EPA's Proposed Guidelines for Carcinogen Risk Assessment as well as the International Agency for Research for Cancer (IARC) criteria for assessing weight of evidence. Using the EPA criteria, the weight of evidence is such that the animal data for carcinogenicity is limited and the epidemiologic data is inadequate. The overall ranking of the weight of evidence is Group C meaning that the substance is a "possible" carcinogen for humans. Using the IARC weight-of-evidence criteria, the animal and epidemiologic data have the same ranking as in the EPA classification, limited and inadequate; however, the overall ranking is considered to be Group 3. An IARC Group 3 classification means that while the evidence may range from limited to inadequate, the overall conclusion is that the data are "inadequate" to assess the human carcinogenic potential. The Agency believes that its ranking criteria are more instructive for public health impact analysis. The Group C (possible carcinogen) classification given to vinylidene chloride is one of five that could be assigned to a substance. The five classifications are: human carcinogen, probable human carcinogen, possible human carcinogen, not classified (inadequate evidence for assessing carcinogenicity), and no evidence of carcinogenicity for humans.

Although vinylidene chloride has only limited carcinogenicity evidence, an upper-bound estimate of incremental cancer risk can be estimated from the kidney adenocarcinoma data in male mice. The development of this risk estimate is for the purpose of evaluating the "what-if" question: If vinylidene chloride is carcinogenic in humans, what is the possible magnitude of the public health impact? Any use of the risk estimates should include a recognition of the weight-of-evidence likelihood for the carcinogenicity of vinylidene chloride in humans. The upper-bound incremental cancer risk is calculated to be $1.16 \times 10^{-3}$ for 1 μg/kg body weight/day for a continuous lifetime exposure to vinylidene chloride under the presumption that vinylidene chloride is carcinogenic in humans. The upper-bound nature of this estimate is such that the true risk is not likely to exceed this value and may be lower. Expressed in terms of relative potency, vinylidene chloride ranks in the third quartile among the 54 suspect or known human carcinogens evaluated by EPA's Carcinogen Assessment Group.

# 2. INTRODUCTION

EPA's Office of Research and Development has prepared this health assessment to serve as a "source document" for Agency use. This health assessment was originally developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of vinylidene chloride under the Clean Air Act. However, the scope of this document has been expanded to address vinylidene chloride in relation to sectors of the environment outside of air. It is expected that this document will be able to serve the information needs of many government agencies and private groups that may be involved in decision-making activities related to vinylidene chloride.

The basic literature search for this document is complete up through August, 1983, with the exception of the mutagenicity section which is complete through December, 1984. However, selected publications have been included in the carcinogenicity section up through 1985.

# 3. PHYSICAL AND CHEMICAL PROPERTIES

The data and the discussion presented below have been obtained from Hushon and Kornreich (1978) and Wessling and Edwards (1970), except in cases where the reference is specifically cited.

## 3.1 SYNONYMS AND TRADE NAMES

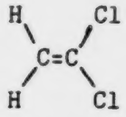
Chemical Abstracts Name: 1,1-dichloroethene

CAS No.: 75-35-4

RTECS No.: KV92750

Synonyms: VDC, 1,1-dichloroethene (1,1-DCE), 1,1-dichloroethylene, vinylidene chloride monomer, vinylidene dichloride

## 3.2 STRUCTURAL AND MOLECULAR FORMULAE AND MOLECULAR WEIGHT



$C_2H_2Cl_2$ Molecular Weight: 96.95

## 3.3 PHYSICAL PROPERTIES

### 3.3.1 Description

Vinylidene chloride is a clear, colorless liquid with a characteristic "sweet" odor.

### 3.3.2 Boiling Point

31.56°C (at 760 mm Hg)

### 3.3.3 Freezing Point

-122.5°C

### 3.3.4 Density

Liquid density at 0°C: 1.2517 g/cm$^3$

Liquid density at 20°C: 1.2132 g/cm$^3$

Vapor density: 3.34 (air = 1)

Density in saturated air: 2.8 (air = 1)

Percent in saturated air: 78%

### 3.3.5 Refractive Index

$n_D^{20}$: 1.42468

### 3.3.6 Solubility

Solubility of vinylidene chloride in water: 2250 mg/l (at 25°C) (Delassus and Schmidt, 1981)

Solubility of water in vinylidene chloride: 0.35 g/l (at 25°C)

Vinylidene chloride is soluble in acetone, ethanol, benzene, diethyl ether, and chloroform.

### 3.3.7 Volatility

Vapor Pressure (P) in mm Hg: $\log P_{mm} = 6.9820 - \frac{1104.29}{t + 237.7}$

(where t = temperature in °C)

Therefore, P = 600 mm Hg at 25°C

### 3.3.8 Volatility from Water

Calculated Henry's law constant (unitless) at 25°C: 7.8 (Dilling, 1977)

Half-life ($t_{1/2}$) for evaporation from water at 25°C for a solution depth of 6.5 cm (Dilling, 1977):

Calculated: 20.1 min

Experimental: 27.2 min

The theoretical equation of Dilling (1977) was derived for a specific hydrodynamic regime and may not be applicable for all waters.

### 3.3.9 Hazard Parameters

Flash point (tag open-cup): 3°F

Flash point (tag closed-cup): -2°F

Autoignition temperature: 1031°F

Explosive limit with air at 28°C

lower: 7.3% by volume

upper: 16.0% by volume

3.3.10 Dielectric Constant

4.67 (at 16°C)

3.3.11 Thermodynamic Data

Latent heat of vaporization at 25°C: 6328 cal/mol

Latent heat of vaporization at boiling point: 6257 cal/mol

Latent heat of fusion: 1557 cal/mol

Specific heat: 0.275 cal/g

Heat of combustion: 261.93 kcal/mol

Heat of polymerization: -18.0 kcal/mol

Heat of formation (liquid monomer): -6 kcal/mol

Heat of formation (gaseous monomer): 0.3 kcal/mol

Heat capacity at 25.15°C (liquid monomer): 26.745 cal/mol/degree

Heat capacity at 25.15°C (ideal gas): 16.04 cal/mol/degree

Critical parameters:

Temperature ($T_c$): 222°C

Volume ($V_c$): 2.19 $cm^3$/mol

Pressure ($P_c$): 51.3 atm

3.3.12 Viscosity

0.3302 cP (centipoise) (at 20°C)

3.3.13 Conversion Factors at 25°C and 760 mm Pressure:

1 ppm $\equiv$ 3.97 $mg/m^3$ (air)

## 3.4 STORAGE AND TRANSPORTATION OF THE MONOMER

Without added inhibitors, vinylidene chloride in the presence of air form complex peroxide compounds at temperatures as low as -40°C. The peroxide is violently explosive. Decomposition products of vinylidene chloride peroxide are formaldehyde, phosgene, and hydrochloric acid. Since the peroxide is a polymerization initiator, formation of insoluble polymer in stored vinylidene chloride monomer may be an indication of peroxide formation. The peroxides are adsorbed on the precipitated polymer. Any dry composition containing more than about 15% peroxide detonates from a slight mechanical shock or from heat; therefore, the separation of the polymer from the monomer by filtration, evaporation, or drying may result in explosion.

Inhibitors are used to prevent formation of peroxides in the monomer. The most common practice to inhibit peroxide formation is the addition of 200 ppm of the monomethyl ether of hydroquinone (MEHQ). Other inhibitors include alkylamines, phenols, and organic sulfur derivatives. Inhibited vinylidene chloride is shipped under a nitrogen blanket to avoid contact with air and to prevent the formation of peroxides. In the absence of water, light, and excessive heat, inhibited vinylidene chloride under a blanket of nitrogen can be stored indefinitely.

To retard polymerization, uninhibited vinylidene chloride should be kept away from light and at a temperature below -10°C. Uninhibited vinylidene chloride can be stored in mild steel, stainless steel, or nickel containers. Contact with copper and aluminum should be avoided because the acetylinic impurities in vinylidene chloride may form copper acetylides and aluminum acetylides, and because aluminum reacts with vinylidene chloride to form aluminum chloroalkyls. These classes of compounds are extremely reactive and potentially hazardous.

## 3.5 CHARACTERISTICS OF THE COMMERCIAL PRODUCT

A typical analysis of commercial-grade vinylidene chloride monomer (excluding inhibitors) is as follows: vinylidene chloride 99.8%; trans-1,2-dichloroethylene, 900 ppm; vinyl chloride, 800 ppm; 1,1,1-trichloroethane, 150 ppm; cis-1,2-dichloroethylene, 10 ppm; and 1,1-dichloroethane, ethylene chloride, and trichloroethylene--each less than 10 ppm.

## 3.6 PURIFICATION OF THE MONOMER

Vinylidene chloride forms an azeotrope with 6% methanol. Distillation of the azeotrope, followed by extraction of the methanol with water, is a method of purification of vinylidene chloride.

## 3.7 CHEMICAL REACTIVITY

Vinylidene chloride, like other olefins, is expected to undergo addition reactions. An example of this addition reaction with HCl is as follows:

$$CH_2{=}CCl_2 + HCl \longrightarrow CH_3{-}CCl_3$$

This reaction is used commercially for the production of 1,1,1-trichloroethane (methyl chloroform).

The addition reaction of vinylidene chloride with bromine is shown below:

$$CH_2{=}CCl_2 + Br_2 \longrightarrow CH_2Br{-}CCl_2Br$$

The reaction product from bromination of vinylidene chloride was used as a confirmatory method for the identification and quantification of the parent compound (Going and Spigarelli, 1977). Other reactions of vinylidene chloride with thiolate (McKinney et al., 1959) and cysteinate (Francis and Leitch, 1957) are also known.

In the presence of polymerization initiators, vinylidene chloride undergoes self-polymerization to form homopolymers, or polymerizes with other compounds--namely, vinyl chloride, alkyl acrylates, and acrylonitrile--to yield copolymers. Copolymers containing various amounts of vinylidene chloride are used as packaging films, carpet backing materials, and modacrylic fibers. When compared with that of other monomers, the reactivity of vinylidene chloride towards polymerization is considered to be average.

# 4. SAMPLING AND ANALYTICAL METHODS

Several methods are available for the measurement of vinylidene chloride in air, water, soil and disposal site samples, as well as in polymers, and as migrated monomer into food-simulating solvents. The analysis of vinylidene chloride in air is more frequently studied because atmospheric air is probably the most prevalent medium of vinylidene chloride contamination. Because of its high volatility, vinylidene chloride lost during its industrial production and processing primarily enters the atmosphere. Moreover, vinylidene chloride originally present in aqueous media is likely to contribute significantly to air contamination as a result of its high volatility from water.

The sampling and analysis of vinylidene chloride in different media are discussed individually in the following sections.

## 4.1 AIR

### 4.1.1 Air Sampling

Three general methods have been used in the past for the collection of vinylidene chloride in air samples: grab sampling, cold-trapping, and trapping in sorbent.

Grab samples can be collected in vacuum bottles or in evacuated syringes (Irish, 1967, cited in Hushon and Kornreich, 1978). The difficulties with this method of collection are twofold. First, since it does not allow sample preconcentration, the detection limit of the method is too high to permit detection of most ambient air levels of vinylidene chloride. Second, the inherent limitation of any grab sampling method is that samples collected by this method will not permit the determination of time-weighted average concentrations.

The inability to collect samples representative of time-weighted average concentrations with the grab sampling method was overcome by the use of a personal sampler that used the critical orifice concept (Williams et al., 1976). The sampler consisted of an evacuated container (<1 torr) equipped with a critical orifice (2 to 5 μm diameter). In such a sampler, the gas flow into the container remains constant as long as a critical flow pattern exists. In order for this to occur, the internal pressure of the container must remain less than half the external atmospheric pressure during sampling. Thus, the length of sampling time depends on both the size of the orifice and the volume of the sample container. For a container of 100 ml volume equipped with a 2 μm orifice, the critical flow will exist for 22 hours. The use of a critical orifice for time averaged sampling has been used in source sampling for many years. The major problem that occurs is blockage of the orifice by particulate matter.

In the cold-trap method, air containing vinylidene chloride is pumped through a cold-trap maintained at a suitable temperature (Irish, 1967, cited in Hushon and Kornreich, 1978). In order to avoid sample loss, the temperature of the cold-trap must be maintained so that the trapped compound has negligible vapor pressure under the applied pressure. The cold-trap method was used by Singh et al. (1981a,b) for the measurement of ambient air concentrations of vinylidene chloride. In this method, sample preconcentration was conducted with 4-inch long stainless steel tubing packed with 100/120 mesh glass beads and maintained at liquid $O_2$ temperature.

The collection of atmospheric vinylidene chloride by sorbent-trapping has been used by several investigators. The advantage of the sorbent-trapping method is that it permits collection of a large volume of air and it concentrates the compound of interest in a small volume of the sorbent. Both liquid and solid sorbents have been used. Gronsberg (1975) bubbled air through pyridine to collect vinylidene chloride. Guillemin et al. (1979) suggested bubbling air through an impinger containing chilled water for the collection of halogenated ethylenes; however, the method was not applied for sampling air and the efficiency of collection was not established. The disadvantage of handling the impingers in the field and transporting them to the laboratory renders this method unattractive.

The trapping of vinylidene chloride in a solid sorbent is a more suitable method for its collection from air samples. Although silica gel was used in the past (Irish, 1967, cited in Hushon and Kornreich, 1978), sorption on activated carbon has been used extensively in recent years for the collection of vinylidene chloride in air. Many investigators (Russell, 1975; Severs and Skory, 1975; Foerst, 1979) used this method for the monitoring of the higher levels of vinylidene chloride present in workplace atmospheres. In only one investigation (Going and Spigarelli, 1977) was this method utilized for monitoring ambient air levels of vinylidene chloride.

The adsorption capacity of vinylidene chloride on activated carbon depends on the characteristics of the carbon (source, particle size, nature of pretreatment) and the amount used. Generally, for short-term sampling (less than 8 hours) sample tubes containing smaller amounts of carbon are used than for long-term sampling (about 24 hours). For example, an increase in the amount of a specific activated charcoal (PCB 12 x 30) from 150 mg to 600 mg allowed an increase in collection time from a minimum of 10 minutes to at least 75 minutes at a flow rate of 1 liter/minute (Severs and Skory, 1975). The effect of adsorption capacity on the nature of activated carbon was also demonstrated by these investigators. Segmentation of the carbon tubes into a front and back section is usually employed for determination of the breakthrough limit during sample collection. If the back section retains more than a certain predetermined percentage of vinylidene chloride, it is taken as an indication that the breakthrough limit has been exceeded.

The sampling unit usually consists of the following components assembled in the order given: probe, charcoal tube, critical orifice, and a battery-operated pump. The tube containing the charcoal is held vertically at a height of 4 to 6 feet from the ground and air is drawn through it at a constant flow rate for a specified length of time. At the end of the sample collection period, the tube is closed with caps and shipped to the laboratory for analysis.

For a known weight and nature of activated carbon, the breakthrough characteristics during sample collection are dependent on several parameters: (1) concentration of vinylidene chloride in air; (2) flow rate of air through the carbon bed; (3) relative humidity of air; and (4) presence of other contaminants in the air. The influence of the first three factors on the breakthrough volume has been thoroughly studied by Foerst (1979). This investigator sampled vinylidene chloride through a 100 mg front section and 50 mg back section of MSA-8 charcoal tubes, and monitored the vinylidene chloride concentration by GC-FID. The results of his experiments showing the dependence of breakthrough volume on the three parameters are given in Table 4-1. It is evident from Table 4-1 that changes in flow rate and concentration of vinylidene chloride have relatively less effect on both the breakthrough volume and loading at breakthrough than do the changes in relative humidity.

The effect of loading on breakthrough volume of Carbosieve-B was also studied by Russell (1975). This investigator stated that "the quantity of the compound of interest collected affects the breakthrough volume very slightly. A pollutant present at 20 ppm will break through at a slightly smaller volume than when at 1 ppm." Relative humidity, however, has a more profound effect on the breakthrough characteristics. A change in relative humidity from 17 to 95% resulted in an almost 90% decrease in both the breakthrough volume and loading capacity. It should be noted that the findings of Russell (1975) regarding the

TABLE 4-1

Breakthrough Volume of Vinylidene Chloride as a Function of Flow Rate, Concentration, and Relative Humidity[a]

| Concentration ($mg/m^3$) | Flow Rate (l/min) | Relative Humidty (%) | Breakthrough[b] Volume (l) | Loading at Breakthrough (μg) |
|---|---|---|---|---|
| Effect of Flow Rate | | | | |
| 13.5 | 1.00 | 87 | 8.9 | 121 |
| 13.4 | 0.20 | 85 | 6.9 | 93 |
| Effect of Concentration | | | | |
| 114 | 1.00 | 95 | 6.0 | 684 |
| 13.5 | 1.00 | 87 | 8.9 | 121 |
| Effect of Humidity | | | | |
| 114 | 0.74 | 17 | 56.0 | 6375 |
| 114 | 1.00 | 95 | 6.0 | 684 |

[a] Source: Foerst, 1979

[b] Volume at which the effluent from the charcoal tubes is equal to 5% of the concentration being sampled.

effect of relative humidity on breakthrough volume are consistent with those of Foerst (1979) as shown in Table 4-1.

It is interesting to point out that the laboratory breakthrough studies done by Going and Spigarelli (1977) employed air with a relative humidity of 39 to 42% for sampling vinylidene chloride through a 2 g bed of Fisher activated charcoal. They concluded that the maximum loading before breakthrough occurs at approximately 2.5% of the weight of total charcoal bed and that, for vinylidene chloride concentrations much lower than 10 ppm (as expected in ambient air), sampling can be performed at 1 liter/minute for 24 hours without exceeding the breakthrough characteristics. While this may be true at 39 to 42% relative humidity, the results of Foerst (1979) indicate that the volume and loading characteristics will drastically change at considerably higher relative humidity. Although the relative humidity during the collection of field samples was not recorded by Going and Spigarelli (1977), light rain was falling during the collection of some samples, which indicates that the relative humidity during field sampling was much higher than 39 to 42%. The field samples collected by Going and Spigarelli (1977) at high relative humidity at a sampling rate of 1 liter/minute for 24 hours might have exceeded the breakthrough characteristics; consequently, some of the results reported by these investigators may be erroneous.

The possibility of using other sorbents for the preconcentration of vinylidene chloride during atmospheric sample collection was studied by Pellizzari and Bunch (1979). They used three sorbents for the evaluation: charcoal, XAD-2, and Tenax GC for the evaluation. It was determined by these investigators that while charcoal and XAD-2 may form traces of halogenated hydrocarbons by in situ reactions of $Cl_2$, olefins, and other pollutants present in the atmosphere, the possibility of artifact formation was minimal with Tenax GC. Therefore, Tenax GC sorbent was used by these investigators for the collection of vinylidene chloride and other volatile organics in the atmosphere. The performance characteristics of Tenax GC as a sorbent for the collection of organics in ambient air were also evaluated by Brown and Purnell (1979). It should be mentioned that although Pellizzari and Bunch (1979) reported minimal artifact formation with Tenax GC, Singh et al. (1979) observed "serious" artifact formation problems with this adsorbent. In addition, Singh et al. (1979) encountered "serious difficulties" in quantitatively adsorbing and desorbing a few specific species with this sorbent. Walling (1984) suggested a sampling approach with Tenax GC that can be utilized to identify the deficiencies in the monitored data due to artifact formation in the Tenax GC bed, inadequate blank correction, and exceeding breakthrough volume during sample collection.

### 4.1.2 Storage of Samples Collected in Solid Sorbent Tubes

The stability of vinylidene chloride collected in charcoal-packed tubes was studied by a number of investigators (Severs and Skory, 1975; Going and Spigarelli, 1977; Foerst, 1979). No loss of vinylidene chloride was detected when the samples in charcoal-packed glass collecting tubes were stored in a refrigerator (2 to 4°C) for 16 to 21 days (Going and Spigarelli, 1977; Foerst, 1979). Similarly, Tenax-GC cartridges stored in cooled culture tubes showed high recoveries with halogenated aliphatic compounds (Pellizzari, 1982; Krost et al., 1982).

### 4.1.3 Analytical Methods

Vinylidene chloride collected by the grab method can be analyzed by three different methods: gas chromatography with various detectors, infrared (IR) analysis, and Laser Stark Spectrometry. In the combined gas chromatography-mass spectrometric (GS-MS) method applied by Grimsrud and Rasmussen (1975), 20 ml samples were flushed onto a SCOT OV-101 chromatographic column temperature-programmed from -60°C to 100°C. The effluents from the GC were passed to a mass spectrometer operated in the single-ion mode. The identity of vinylidene chloride was established on the basis of chromatographic retention time and by monitoring ions of masses 61 and 96 in the mass spectrum. The detection limit of the method was 5 ppt; however, the chromatographic conditions employed could not separate vinylidene chloride from its isomers (cis- and trans-1,2-dichloroethylene).

A portable IR analyzer was used by Foerst (1979) for continuously monitoring vinylidene chloride produced in a dynamic flow vapor generator. A Wilks Miran IA IR analyzer was used in the absorbance mode at a wavelength of 9.1 μm, with a cell path length of 20.25 meters, and a slit width of 2 mm. The response time for vinylidene chloride was 4 seconds. This method, however, is unsuitable for monitoring vinylidene chloride in atmospheric air for two reasons. First, the concentration of vinylidene chloride in atmospheric air (on the order of ppt) is far below the detection limit of the IR analyzer. Second, some of the other halohydrocarbons present in atmospheric air samples will produce interference in the vinylidene chloride determinations.

Laser Stark Spectrometry with a 9.6 μm line from a $CO_2$ IR laser can be used for the determination of vinylidene chloride; however, the detection limit of this method with a 40 cm Stark cell is in the ppm range (Sweger and Travis, 1979). A multipass Stark cell could result in a significant increase in vinylidene chloride detection sensitivity but is not yet available. Other disadvantages of this method include interferences from other halohydrocarbons such as Freons, methyl chloride, methyl fluoride, and vinyl chloride (Sweger and Travis, 1979).

Both the IR and Laser Stark Spectrometry methods have the inherent capability for continuous monitoring of vinylidene chloride levels in the workplace where the concentration of vinylidene chloride is considerably higher than that of atmospheric air and where few interfering substances may be present.

A modified commercial nitrogen oxide analyzer with an ethylene supply system substituted for the ozone source may also be used for the monitoring of compounds containing unsaturated double bonds (Hilborn et al., 1976). This instrument, which utilizes the principle of chemiluminescence, may be useful for monitoring total unsaturated hydrocarbons in the atmosphere (including vinylidene chloride), but is not suitable for monitoring individual unsaturated hydrocarbons when they are present in a mixture. In addition to detecting olefinic compounds, the instrument also responds to compounds containing sulfide and amine groups (Hilborn et al., 1976).

In one of the more recent methods for determining vinylidene chloride in the ambient air, Singh et al. (1981a,b) collected the samples by cryogenic cooling (see Section 4.1.1). With this method, the desorption of chemicals from the cooling trap was accomplished by maintaining the trap at boiling water temperature and purging it with carrier gas. The desorbed vinylidene chloride was analyzed by a long (10 meter) packed column and electron capture detector.

Gronsberg (1975) used a calorimetric method to quantify vinylidene chloride collected in an impinger containing pyridine. The pyridine solution containing vinylidene chloride was heated with NaOH in a sealed container and then coupled with barbituric acid or aniline in acetic acid to form a cyanine dye. The dye color intensity was measured with a spectrophotometer. Neither vinyl chloride nor acrylonitrile interfered with vinylidene chloride determination. The detection limit of the method (10 $mg/m^3$ of air), however, renders it unsuitable for monitoring vinylidene chloride in ambient air.

Vinylidene chloride collected by bubbling air through water in an impinger can be analyzed by injecting the aqueous solution onto a steam-modified gas-solid chromatograph (Guillemin et al., 1979). The details of this technique are given in Section 4.2.2. A Spherosil XOA-400 column operated at an isothermal temperature of 128°C with a mobile phase containing a mixture of 50 ml/minute $N_2$ gas and 16 ml/minute steam has been successfully used for the separation and quantification (by FID) of the haloethylenes including vinylidene chloride (Guillemin et al., 1979). By using a 200 µl aqueous solution injection with this method, 20 ppb vinylidene chloride in water can easily be detected.

Vinylidene chloride collected by adsorption on solid sorbents is analyzed by a two-step method. The first step in the analysis is the desorption of vinylidene chloride from the sorbent. The second step is the separation, identification, and quantification of the desorbed vinylidene chloride, usually by GC-FID.

Thermal elution and solvent extraction are two commonly used methods for the desorption of vinylidene chloride from solid sorbents. The thermal desorption technique was used by several authors (Pellizzari and Bunch, 1979; Bozzelli et al., 1980). In one thermal desorption method, the sampling tube was reversed (the end where air entered during sampling was attached to the injection port) to allow backflushing of the sorbed compounds into the injection port of a GC. With a 2 x 0.25 inch tube packed with Carbosieve-B, Russell (1975) determined the recovery of vinylidene chloride to be 100% (±3% standard deviation) when the tube was heated at 270°C for 5 minutes at a nitrogen flow of 20 ml/minute. When the dimension of the collection tube was increased to 5.5 by 0.25 inches, however, Severs and Skory (1975) demonstrated that 10 liters of $N_2$ gas were required to pass through a heated (430°C) PCB 12 x 30 activated carbon collection tube to desorb vinylidene chloride. The desorption efficiency under the above conditions was determined to be 88%.

The sensitivity of the activated carbon adsorption and thermal desorption technique is approximately 1 ppb for a 1-liter air sample using FID (Russell, 1975). The high temperature required for desorption, however, may cause some organic compounds to chemically react on the catalytic surface of the sorption tube, thereby altering their chemical composition. For example, methyl bromide appears to exchange a Br atom for a Cl atom (present as an impurity in the adsorbent) during thermal desorption from Carbosieve-B. Thus, peaks from both methyl bromide and methyl chloride appear in the chromatogram (Russell, 1975).

In some of the more recent methods that used Tenax GC as the sorbent, the thermally desorbed vinylidene chloride was either collected in stainless steel tubes under pressure (Bozzelli et al., 1980) or the desorbed gas purged with helium was collected into a liquid nitrogen-cooled nickel capillary trap (Pellizzari and Bunch, 1979). In both cases, the final separation and analysis of vinylidene chloride was done by high resolution GC-FID.

Desorption by solvent extraction has been used by a few investigators. In this technique, the adsorbent is added to 1 to 10 ml $CS_2$ either at room temperature in a crimp-sealed vial (Foerst, 1979), or cooled in wet ice (Severs and Skory, 1975), or in dry ice (Going and Spigarelli, 1977). The effect of $CS_2$ both at 30°C and at -78°C was that almost the same recovery resulted for desorption times ranging from 15 to 90 minutes. The $CS_2$ desorption efficiency was determined to be 100% (Severs and Skory, 1975; Going and Spigarelli, 1977). Foerst (1979), however, reported that the desorption efficiency was dependent on loading level. There was a trend towards lower desorption efficiencies at lower loadings. This dependence of desorption efficiency on loading level is shown in Figure 4-1. The stability of dilute vinylidene chloride solutions in $CS_2$ was studied by Going and Spigarelli (1977). About 20% loss of vinylidene chloride was observed whether the sample was stored at -18°C (freezer), 4°C, or 25°C for 7 days. The loss was much less (about 6%) when the solution was stored for only 4 days at the same three temperatures. Therefore, the desorbed vinylidene chloride in $CS_2$ should be analyzed within 4 days, regardless of the temperature of storage.

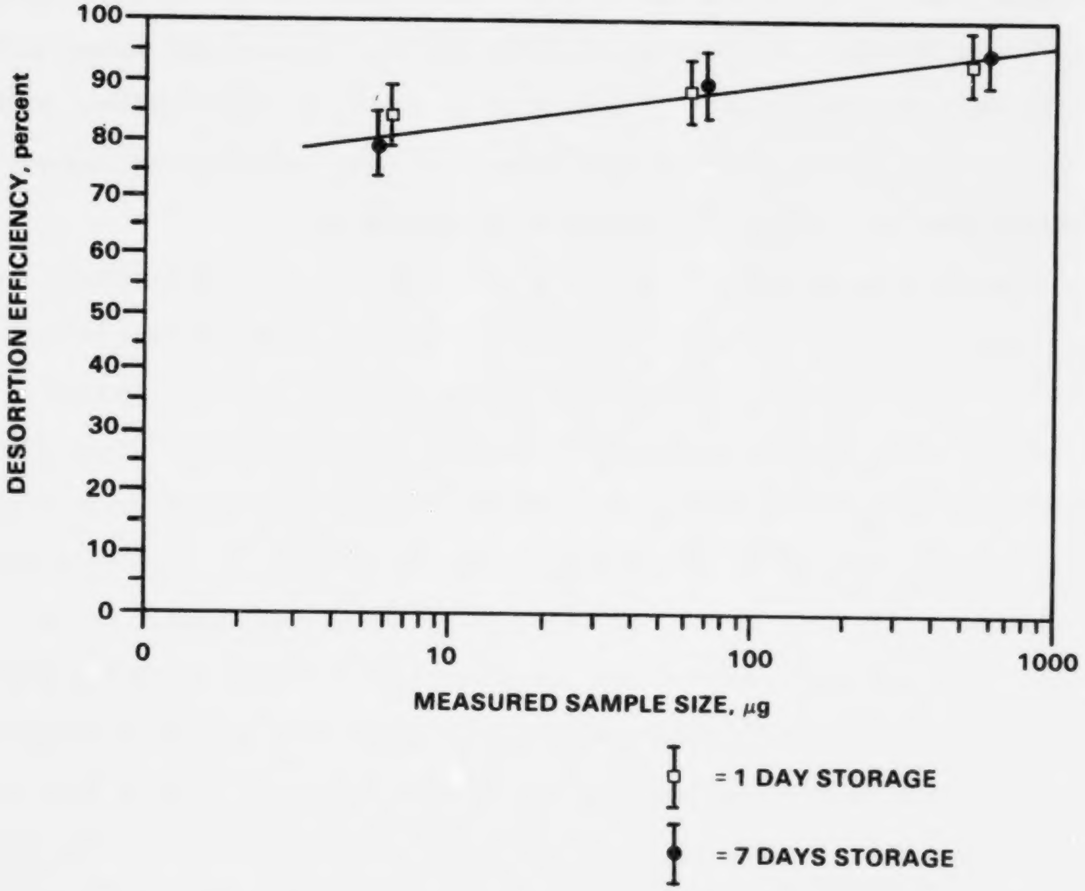


Figure 4-1. Desorption efficiency vs. amount found by GC analysis.

Source: Foerst (1979).

The second step in the analysis of vinylidene chloride by the solvent extraction method involves injection of the $CS_2$ solution into a GC-FID. The choice of FID is based upon the fact that $CS_2$ has very little response with FID. A number of columns including 10% TCEP, OV-225, 3% Dexsil, Tenax GC, Porapak N, and Chromosorb 102 were tested (Foerst, 1979). None of these columns were able to separate vinylidene chloride, vinyl chloride, and $CS_2$. The most suitable column was found to be Durapak OPN on Porasil C (Foerst, 1979; Going and Spigarelli, 1977). Limited success was attained with 20% DC200 (Severs and Skory, 1975), but the separation of vinylidene chloride from $CS_2$ solvent was less than ideal with this packing material.

The confirmation for vinylidene chloride quantified by FID was made both by mass spectrometry and by bromination of vinylidene chloride (Going and Spigarelli, 1977). The bromination was performed by reacting vinylidene chloride with a 500-fold molar excess of $Br_2$ for 30 minutes. The excess $Br_2$ was removed by 0.5 M NaOH solution. The brominated vinylidene chloride was dried over anhydrous $Na_2SO_4$ and analyzed on a 1.5% OV-101 column equipped with an FID.

## 4.2 WATER

### 4.2.1 Water Sampling

Grab water samples should be collected in narrow-mouth, glass, screw-cap bottles with Teflon-faced, silicone rubber, septa cap liners (Federal Register, 1979; Going and Spigarelli, 1977; Bellar and Lichtenberg, 1979;Bellar et al., 1979). To avoid vinylidene chloride loss, the bottles should be filled to overflow with water and should be sealed immediately without leaving any head-space. If the sample contains free or combined chlorine, sodium thiosulfate (10 mg/40 ml for 5 ppm $Cl_2$) should be added to the water at the time of sample collection (Federal Register, 1979; Bellar et al., 1979). If left unneutralized, $Cl_2$ will react with organics in water and will increase the levels of chlorinated hydrocarbons (Bellar et al., 1974, 1979). The samples collected this way should be shipped in ice and refrigerated during storage. A sample stored at 4°C was found to be stable for at least 9 days (Going and Spigarelli, 1977) and samples similarly stored for up to 27 days were stable (Bellar and Lichtenberg, 1981). It was suggested that the analysis of the stored samples be performed within 14 days (Federal Register, 1979).

### 4.2.2 Analytical Methods

Vinylidene chloride in water samples has been analyzed by three different techniques: direct head-space analysis, direct injection on a steam-modified gas-solid chromatograph, and by the purge-trap technique.

The direct head-space analysis studied by Piet et al. (1978) has the advantage that no preconcentration and prehandling of the samples are required; therefore, systematic error is reduced. The additional advantages of the method are its good GC separability due to the absence of solvents and its low detection limit. The method can be applied for tap water, ground water, and surface waters. In this method, 100 ml $N_2$ is bubbled through 1 liter of water at 30°C and the $N_2$ containing the head-space vinylidene chloride is trapped between two columns of water in a special apparatus (Piet et al., 1978). Up to 500 µl of the head-space gas could be injected into a GC equipped with an electron capture detector. The GC column used was a wide-bore glass capillary coated with OV-225 and operated isothermally at 22°C. The detection limit for a 100 µl injection was determined to be 2 µg/l.

Castello et al. (1982) also used the head-space technique for the determination of halocarbons in drinking water. In their technique, a 5 ml aliquot of water was placed in a closed vial, the sample was thermostatically held at 30°C for 1 hour in a water bath, and 200 µl of the head-space gas was injected into the GC column attached to an electron capture detector. The authors did not provide the detection limit for vinylidene chloride by this method.

The steam-modified gas-solid chromatography technique, which utilizes a composite of inert carrier gas and a controlled amount of steam as the mobile phase, a porous packing material as the stationary phase, and a FID, appears to be a powerful tool for the analysis of aqueous samples (Guillemin et al., 1979). For the analysis of aqueous samples of haloethylenes including vinylidene chloride, a Spherosil XOA-400 column operated isothermally at 128°C and a mobile phase containing a mixture of 50 ml/minute $N_2$ and 16 ml/minute steam were sucessfully used. One advantage of this method is that it allows for direct aqueous injections, thereby eliminating time-consuming and loss-incurring pretreatment of samples. The other advantage of this technique over the traditional GC technique is that large amounts of aqueous samples can be injected onto the column without causing any fluctuation in the baseline, even at maximum sensitivity of the detector amplifier (Guillemin et al., 1979). By using a 200 µl injection with this method, 20 ppb vinylidene chloride in aqueous samples can easily be detected. The method may not be applicable to polluted water samples, however, because of GC separation problems arising from co-contaminants and rapid deterioration of the GC column due to sorption of impurities.

The purge-trap technique is the most widely applied method for the analysis of vinylidene chloride in aqueous samples (Bellar et al., 1979; Bellar and Lichtenberg, 1974, 1979; Going and Spigarelli, 1977; Dowty et al., 1975). This method is applicable for vinylidene chloride analysis in a diversity of media, including drinking water on one extreme, and raw undiluted sewage on the other (Bellar et al., 1979). This technique has been adopted as the recommended method for the analysis of vinylidene chloride in water samples (Federal Register, 1979). In the purge-trap technique, the water sample is purged with an inert gas, such as $N_2$ or He, at room temperature. The purged vinylidene chloride is adsorbed on a trapping column. The trapped vinylidene chloride is thermally desorbed and passes through an analytical column.

The optimum conditions for purging were determined to be either 38 ml/minute for 10 minutes (Going and Spigarelli, 1977) or 40 ml/minute for 11 to 15 minutes (Bellar and Lichtenberg, 1979). Increasing the purge time at the specified flow rate of inert gas resulted in a decrease in vinylidene chloride recovery (Going and Spigarelli, 1977). The purged vinylidene chloride was trapped in a Tenax GC precolumn (Going and Spigarelli, 1977; Bellar et al., 1979). The thermal desorption was performed in the backflush mode for 4 minutes at a temperature of 180°C with an inert gas flow rate of 20-60 ml/minute (Bellar et al., 1979). The average recovery of vinylidene chloride determined for the entire method by multiple analyses over 21 days using repeated 5 ml aliquots of 2 µg/l aqueous solution was determined to be 88% by this purge-trap thermal desorption technique (Bellar and Lichtenberg, 1979). The sensitivity of this technique is dependent on the method of detection and the volume of water used for purging. However, the method is very sensitive because the entire amount of purged vinylidene chloride is used for a single analysis. A method detection limit (MDL) of 0.003 µg/l was reported in EPA method 502.1 (Bellar and Lichtenberg, 1981).

Analytical columns packed with four kinds of materials--0.2% Carbowax 1500 on 80/100 mesh Carbopack-C (Bellar and Lichtenberg, 1979; Going and Spigarelli, 1977), 1% SP-1000 on 60/80 mesh Carbopack-B (Federal Register, 1979), *n*-octane on 100/120 mesh Porasil-C (Federal Register, 1979), and 10% SF-96 and 1% Igepal CO 880 capillary column (Dowty et al., 1975)--were found to be best suited for the separation of vinylidene chloride from co-contaminants. Proper temperature programming was found to be necessary for successful separation.

Both flame ionization (Dowty et al., 1975; Going and Spigarelli, 1977) and halogen specific (Bellar et al., 1979; Federal Register, 1979) detectors were used for the quantification of vinylidene chloride. The FID is somewhat unsuitable, however, because the calibration procedure needed with the purge-and-trap method requires the preparation of aqueous standards by mixing reagent-free water with aliquots of standard solution in water-miscible solvents, such as methanol. Consequently, the aqueous standards prepared in this manner contain relatively large quantities of the miscible organic solvent, relative to the compound of interest. While these organic solvents are purged with relatively low efficiency, their relatively high concentration may lead to significant interferences with the FID. Therefore, the halogen specific detector is more suitable for the purge-trap technique.

The confirmation of vinylidene chloride quantified by GC-FID or GC-Hall detector was made by the GC-MS technique (Bellar et al., 1979; Going and Spigarelli, 1977; Dowty et al., 1975; Federal Register, 1979). Mass spectrometry, either in the specific ion mode (SIM) or in the mass scan mode, was used as the confirmatory technique. The specific ion mode, however, showed better sensitivity of detection (Bellar et al., 1979).

The purge-trap technique described in the preceding discussions, or slightly modified methods, have been used by several investigators recently for the determination of vinylidene chloride in aqueous samples (Pereira and Hughes, 1980; Otson et al., 1982a,b). The precision of the purge-trap method using GC/MS also has been determined recently by Olynyk et al. (1981).

## 4.3 SOIL AND DISPOSAL SITE SAMPLES

### 4.3.1 Sampling

DeLeon et al. (1980) reported taking samples from 30-foot vertical borings using the split-spoon method. The samples were placed in glass jars, sealed with Teflon-lined screw caps, and shipped in containers maintained at temperatures between 6°C and 10°C. Upon arrival at the laboratory, the samples were transferred to a freezer, where they were kept at -20°C until analyzed. Speis (1980) collected sediment with a methanol-rinsed stainless steel spatula in 40 ml vials sealed with teflon baked silicon septa. The vials were filled to capacity to minimize headspace and stored up to 1 week at wet ice temperatures.

### 4.3.2 Analysis

DeLeon et al. (1980) solvent extracted the samples in sealed vials with either n-hexane (for FID quantification) or n-hexadecane (for MS confirmation) by mixing them with a vortex for 30 seconds. Tribromomethane (bromoform) was added as an internal standard for the samples analyzed by FID. The solvent extracts were quanitified for vinylidene chloride by the GC-FID method and by GC coupled with MS for confirmation. A temperature-programmed capillary column coated with SE-52 was employed as the analytical column. This column (27 meters long) was found to be suitable for the separation of vinylidene chloride from its isomers cis- and trans-1,2-dichloroethylene. The recoveries of vinylidene chloride by this method were determined at different levels of contamination. At vinylidene chloride levels of 10 µg/g, 100 µg/g, and 300 µg/g, the percent recoveries were determined to be 110.5 ± 3.5 (standard deviation), 86.9 ± 8.3, and 80.2 ± 3.7, respectively. The detection limit of the method was determined to be 10 µg/g.

Speis (1980) transferred a weighed portion of sediment into a Hypo vial, sealed the vial, and injected internal standards. The vials were then placed in a sand bath at 110°C and purged with an inert gas at 40 ml/min by means of a sharpened stainless steel tube inserted through the septum to the bottom of the vial. The outlet was passed through a water trap to a Tenax silica gel trap. Desorption and analysis were virtually identical to EPA Method 624. Detection limits were reported to be 5 µg/kg and recoveries were 67% for a 10 µg spike, 90% for a 20 µg spike, 81.1% for a 40 µg spike, and 84% for an 80 µg spike.

## 4.4 MONOMER CONTENT IN POLYMERS AND MONOMER MIGRATION INTO FOOD-SIMULATING SOLVENTS

Saran film is a copolymer of vinyl chloride (15-20%) and vinylidene chloride (80-85%). It is marketed primarily as household film (0.5 mil thickness) and as industrial film (2.0 mil thickness). The monomers in saran films used for food packaging applications have the potential for migration into foods. Therefore it is important to determine the monomer content in the polymer and the extent of the monomer's migration into food-simulating solvents.

### 4.4.1 Sample Collection

The saran films required for analysis of residual monomer or of its migration into food-simulating solvents can be obtained from the manufacturer or can be purchased directly at retail stores.

### 4.4.2 Analysis

The analysis of saran films for the monomer concentration has been accomplished by two methods. In one method (Birkel et al., 1977; Hollifield and McNeal, 1978), the film was dissolved in tetrahydrofuran (THF) in a teflon-lined screw cap glass bottle at 70°C. The dissolved polymer containing the monomer was directly injected into a GC equipped with an electron capture detector for quantification. The analytical column was packed either with Chromosorb-102 (Birkel et al., 1977) or Chromosorb-104 (Hollifield and McNeal, 1978). When THF spiked with vinylidene chloride was used, this method yielded recoveries of 96-102% through the quantification steps (Birkel et al., 1977). When a Chromosorb-104 column was used, the detection limit of the method was determined to be about 1 ppm vinylidene chloride in the polymer (Hollifield and McNeal, 1978). The confirmatory technique used with gas-solid chromatography-electron capture detectors (GSC-ECD) was GC-MS in the specific ion mode (SIM). The disadvantage of this method is that the injections of polymer solutions leave polymer residue in the glass liner of the injection port. If the injection port liner is not cleaned frequently, the polymers may enter the column and alter its performance.

This disadvantage in the analysis of vinylidene chloride monomer by solvent dissolution of the polymer can be overcome by analyzing the monomer content of the polymer by the head-space technique. The additional advantage of the head-space technique is that the method is free from solvent interference such as that encountered with the solvent dissolution method. The head-space technique was used by Going and Spigarelli (1977). In this method, the polymer was crimp-sealed in a vial (teflon-lined cap) and the vial was heated. The optimum conditions for heating were determined to be 100°C for 90 minutes. At a higher temperature (150°C), vinylidene chloride was found to decompose; at lower temperatures (50°C and 75°C), vinylidene chloride was not released from the polymer. A 100 µl head-space gas was injected into a Durapak OPN on an 80/100 mesh Porasil-C GC column and the separated vinylidene chloride was quantified by FID. This technique, however, has its own disadvantage. The calibration of the method requires standard polymer samples that have known vinylidene chloride concentration. In the absence of such a standard, it becomes impossible to determine the recovery of vinylidene chloride from the thermal release procedure used in the head-space technique.

In order to determine the extent of the migration of vinylidene chloride, the analysis of food-simulating solvents in contact with polymers was performed by Hollifield and McNeal (1978). They used three food-simulating solvents--corn oil, heptane, and water. Strips of saran wrap were placed in 400 ml food-simulating solvent in a sealed can and stored at 49°C; this temperature is suggested in the Code of Federal Regulations (CFR, 1977, cited in Hollifield and McNeal, 1978) as the most severe temperature condition to which the food packaging material is expected to be subjected. After the desired storage time, 20 ml of the solvent were placed in screw cap bottles sealed with teflon-lined septa. The bottles were heated to 90°C for 30 minutes or more and 1-2 ml of the head-space gas was withdrawn for analysis. For canned water samples, the head-space gas at 25°C was analyzed. The quantitative analysis was done by a GC with a Chromosorb-104 column and an ECD. A GC equipped with the same column and coupled with a mass spectrometer operated in the selective ion mode was used as a confirmatory analysis. The recovery of vinylidene chloride after spiking the migration-system-solution and analyses by the head-space gas-solid chromatography technique was found to be quantitative (95 to 138%). The vinylidene chloride detection limit was determined to be 5-10 ppb in the food-simulating solvents (Hollifield and McNeal, 1978).

A similar head-space technique was employed by Gilbert et al. (1980) for the determination of vinylidene chloride monomer in packaging films. In this method, narrow strips of film were put into hypovials and sealed with rubber septa. The hypovials were heated to 120°C by an air-circulating fan oven for 30 minutes and 1 ml of the head-space gas was removed for analysis by GC with an ECD.

## 4.5 ANALYSIS OF FOODS AND OTHER BIOLOGICAL SAMPLES

The analysis of vinylidene chloride in food products in contact with polyvinylidene chloride wrappings was performed by Gilbert et al. (1980) by a head-space method similar to that described for wrapping materials. For cooked meat and cheese products, the samples were ground under liquid nitrogen to a fine powder prior to their placement into hypovials. The hypovials were heated to 60°C in a waterbath for a minimum of 90 minutes prior to analysis.

The analysis of vinylidene chloride in fish samples and in body tissues was performed by the purge-trap method similar to that described for aqueous samples (Lin et al., 1982; Easley et al. ,1981). Lin et al. (1982) used computerized

GC-MS for the determination of vinylidene chloride in fish, and Easley et al. (1981) used a GC equipped with a Hall electrolytic conductivity detector for vinylidene chloride determination in body tissues. The approximate limit of detection for vinylidene chloride in spiked fish samples was determined to be 10 µg/kg by Easley et al. (1981). Lin et al. (1982) determined the absolute detection limit of 50 µg/kg for the analysis of vinylidene chloride in body tissues by their method.

Hiatt (1981, 1983) analyzed fish tissue for volatile compounds including vinylidene chloride by vacuum distillation. Whole fish, stored in dry ice, were homogenized in a food cutter with added liquid nitrogen to prevent loss of volatiles. A 10 g fish sample in the sample flask was then heated to 50°C at 360 torr while immersed in an ultrasonic bath. Distillation was continued for 15 min.; the volatiles were trapped at liquid nitrogen temperature. The contents of the trap were transferred to a DB5 coated fused-silica capillary column (FSCC) (30 m x 0.32 mm, 0.1 µm film thickness) at -99°C, which was then programmed up to 180°C. Analysis was performed by mass spectrometry. Recovery was reported to be 74 ± 8% as opposed to 59 ± 19% for purge and trap of a diluted sample. FSCC chromatography gave better resolution than packed columns for late-eluting peaks.

# 5. SOURCES IN THE ENVIRONMENT

The purpose of this document is to present available information relevant to human health effects that could be caused by this substance.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exosure to this substance.

Vinylidene chloride is a man-made chemical. To date, there is no evidence that any natural process produces this chemical. The sources of vinylidene chloride in four different environmental media--air, water, soil, and foods--are discussed below.

## 5.1 SOURCES OF AIR POLLUTION

The following operations contribute to the presence of vinylidene chloride in the air:

(1) manufacture of vinylidene chloride monomer;

(2) manufacture of polymers containing polyvinylidene chloride;

(3) processing or fabrication of polyvinylidene homopolymers and copolymers;

(4) storage, handling, and transportation of the monomer;

(5) chemical intermediate production; and

(6) incineration of polymers containing polyvinylidene chloride.

### 5.1.1 Manufacture of Vinylidene Chloride Monomer

Vinylidene chloride is manufactured industrially either as a coproduct of ethylene dichloride, produced from the chlorination of ethane or ethylene, or by the dehydrochlorination of 1,1,2-trichloroethane. The chemical reactions for the processes are the following:

$$CH_3\text{-}CH_3 + 3Cl_2 \longrightarrow CH_2{=}CCl_2 + 4HCl$$

$$CH_2{=}CH_2 + 2Cl_2 \longrightarrow CH_2{=}CCl_2 + 2HCl$$

$$CH_2Cl\text{-}CHCl_2 + NaOH \longrightarrow CH_2{=}CCl_2 + NaCl + H_2O$$

The current domestic manufacturers of vinylidene chloride monomer and their estimated annual capacity are given in Table 5-1.

The Lake Charles facility of PPG Industries, which used 130 million pounds of vinylidene chloride annually for captive consumption in the manufacture of 1,1,1-trichloroethane, now produces 1,1,1-trichloroethane by a route that does not utilize vinylidene chloride (PPG Industries verbal communication, 1980). The Lake Charles facility of PPG continues to produce vinylidene chloride, however, for sale in the merchant market.

It is reported that the Dow facility at Freeport produces about two-thirds of its total annual capacity (Neufeld et al., 1977). Dow captively consumes about 85% of the total vinylidene chloride it manufactures annually. The balance is shipped to its other polymer-producing facilities or sold to the merchant market.

Vinylidene chloride is emitted to the air during the manufacture of the monomer. The air emission factors, that is, the fraction of vinylidene chloride lost to the air during its manufacture, can be estimated from material balance.

TABLE 5-1

Monomer Production Facilities in the United States and Their Capacities[a]

| Manufacturer | Location | Estimated Capacity (million lb/yr) |
|---|---|---|
| PPG Industries | Lake Charles, LA | 78[b] |
| Dow Chemical | Freeport, TX and Plaquemine, LA | 95-100 |

[a] Source: Neufeld et al., 1977

[b] Figure obtained from Anonymous, 1978

TABLE 5-2

Air Emissions of Vinylidene Chloride During Monomer Manufacture[a]

| Producer | Location | Air Emission Factor (emission in lb/100 lb monomer produced) | Annual Emission (thousands of lb/yr) |
|---|---|---|---|
| PPG Industries | Lake Charles, LA | 0.10 | 62 |
| Dow Chemical | Freeport, TX and Plaquemine, LA | 0.48 | 384 |

[a] Source: Neufeld et al., 1977

These air emission factors for vinylidene chloride manufacturing processes are given by A.D. Little, Inc. (1976, cited in Going and Spigarelli, 1977). Based on these values and the estimated production (80% of capacity) of vinylidene chloride monomer, the annual emission rates derived are shown in Table 5-2.

The total estimated emission of 450,000 pounds/year is considerably lower than the figure given by other authors (Hushon and Kornreich, 1978). The difference is due mainly to PPG Industries' no longer producing 1,1,1-trichloroethane from vinylidene chloride. However, PPG still produces vinylidene chloride for sale to polymer producing facilities.

According to the unpublished data submitted by Dow Chemical (Dow, 1984), the estimated yearly emission of vinylidene chloride from its thermal recovery units that handle the reactor waste streams is 820 lbs. for both plants. Dow also estimates (Dow, 1984) 10,000 lbs/yr for fugitive emissions from both monomer and polymer manufacturing facilities. Based on Dow estimate, the total vinylidene chloride emission by its monomer production facilities is 4820 lbs/yr assuming 4000 lbs/yr for fugitive emission from its monomer production facilities.

### 5.1.2 Manufacture of Polymers Containing Polyvinylidene Chloride

The two primary uses of vinylidene chloride are in the manufacture of polymers and chemical intermediates. The breakdown of vinylidene chloride consumption in different industrial uses is given in Table 5-3.

It can be seen from Table 5-3 that except for a small application as a chemical intermediate and some export, most of the vinylidene chloride is consumed domestically for the production of polymers. Because of its excellent fire and barrier resistance properties, vinylidene chloride polymer has been used in the manufacture of copolymers with other monomers. In general, copolymers that contain a high percentage of vinylidene chloride (70-95%) are

TABLE 5-3

Yearly Consumption of Vinylidene Chloride in Different Industries[a]

| Industry | Amount consumed (million lb/yr) | Percent of Total |
|---|---|---|
| Polymers | | |
| Domestic Use | 108-115 | 81% |
| Export | 20 | 15% |
| Chemical Intermediate | 5 | 4% |
| TOTAL | 133-140 | 100% |

[a] Source: Neufeld et al., 1977

TABLE 5-4

Polymerization Processes, Products, and Their Applications[a]

| Process | Product | Application |
|---|---|---|
| Emulsion polymerization | Emulsion latex<br>Speciality latex | Barrier coating<br>Carpet backing |
| Suspension polymerization | Resin | Barrier coating on multilayer laminate<br>Molded plastics<br>Extruding films and pipes |
| Solution polymerization | Fiber | Modacrylic fibers |

[a] Source: Neufeld et al., 1977

used for the barrier resistance characteristic, and copolymers with a lower percentage of vinylidene chloride (10-40%) are used for fire retardant properties. The three different polymerization processes used industrially for the production of polymeric materials and the uses of these polymers are shown in Table 5-4.

Emulsion latexes used as barrier coatings usually contain 70 to 95% vinylidene chloride copolymerized with a variety of other monomers such as vinyl chloride, acrylic acid, and acrylonitrile. These copolymers have a unique and excellent barrier resistance to gases, water vapor, organic vapors, and odors. The speciality latexes obtained by copolymerization of 10 to 40% vinylidene chloride and butadiene-styrene have excellent fire retardant properties and are used as carpet backing materials.

The solid resins obtained by converting emulsion or suspension latexes to the powdered resin may contain different amounts of vinylidene chloride. As in the case of latexes, the characteristics of the copolymer are strongly influenced by the vinylidene chloride content. Solid resins are used for coating cellophane and extrusion into film or for manufacturing multilayer laminates.

Solution polymerization of 10 to 30% vinylidene chloride with acrylonitrile produces modacrylic fibers. The modacrylics are produced solely for the flame retardant characteristics they impart to sleepwear fabric, drapery fabric, and automobile upholstery.

The major plant sites for vinylidene chloride polymerization and the type of polymer produced by these facilities are shown in Table 5-5.

The estimated annual emissions of vinylidene chloride from polymer synthesis are given in Table 5-6.

TABLE 5-5

Polymerization Sites and Type of Polymer Produced[a]

| Manufacturer | Location | Polymer type | Primary usage |
|---|---|---|---|
| Dow Chemical | Midland, MI | Suspension latex | Barrier coating |
| | | Emulsion latex | Barrier coating |
| | | Solid resin | Barrier coating |
| | Dalton, GA | Speciality latex | Carpet backing |
| W.R. Grace | Owensburg, KY | Emulsion latex | Barrier coating |
| | | Speciality latex | Carpet backing |
| Morton Chemicals | Ringwood, IL | Emulsion latex | Barrier coating |
| A.E. Staley | Lemont, IL | Emulsion latex | Barrier coating |
| Rohm and Haas | Knoxville, TN | Emulsion latex | Barrier coating |
| GAF | Chattanooga, TN | Emulsion latex | Barrier coating |
| | | Speciality latex | Carpet backing |
| Reichhold Chemicals | Cheswold, DE | Emulsion latex | Barrier coating |
| | | Speciality latex | Carpet backing |
| National Starch | Meridosia, IL | Emulsion latex | Barrier coating |
| | | Speciality latex | Carpet backing |
| Eastman | Kingsport, TN | Modacrylics | Fiber |
| Monsanto | Decatur, AL | Modacrylics | Fiber |
| American Cyanamid | Pensacola, FL | Modacrylics | Fiber |
| DuPont | Circleville, OH | Solid resin | Barrier coating |

[a] Source: Neufeld et al., 1977

TABLE 5-6

Estimated Annual Emissions of Vinylidene Chloride from Polymer Synthesis[a]

| Polymer | Emission Factor (lb/100 lb vinylidene chloride polymerized) | Annual Emissions (thousands of lb/yr) |
|---|---|---|
| Barrier-coating latex | 0.60 | 120 |
| Latex for miscellaneous coatings | 1 | 150 |
| Synthetic fibers | 1 | 160 |
| Resin for cellophane coating | 0.7 | 182 |
| Extrusion resin (emulsion) | 0.13 | 27 |
| Extrusion resin (suspension) | 0.26 | 40 |
| TOTAL | | 679 |

[a] Source: A.D. Little, Inc., 1976

### 5.1.3 Processing or Fabrication of Polymers

The fabrication processes include coating of paper, glassine, paper products, cellophane, and plastic films. Fabrication processes also include extrusion of films and monofilament fibers and molding operations.

The major polyvinylidene chloride latex users for miscellaneous fabrication processes are given in Tables 5-7, 5-8, and 5-9.

The estimated annual emission rate of vinylidene chloride in the processing of polyvinylidene chloride polymers is given in Table 5-10. However, according to the unpublished data submitted by Dow Chemical (Dow, 1984), the emission of vinylidene chloride from its polymer production facilities ranges from 172,000 to 182,000 lbs/yr.

### 5.1.4 Storage, Handling, and Transportation of the Monomer

Vinylidene chloride monomer is shipped from the production sites to the user sites in bulk railroad tank cars or tank trucks. A small unspecified quantity is shipped in drums (Neufeld et al., 1977). Approximately 90 million pounds of vinylidene chloride are transported annually in various ways. Vinylidene chloride emissions can be expected to occur during storage, transfer, and filling operations. It has been estimated that of the total vinylidene chloride loss during its manufacture, subsequent polymerization, and processing, 25% takes place during storage, transfer, and handling and 75% takes place during processing (Neufeld et al., 1977). Since only half of the total vinylidene chloride manufactured is transported to other locations (the other half is captively used), this transported half is solely responsible for losses related to storage, transportation, and handling. The total vinylidene chloride loss during manufacture, subsequent polymerization, and processing adds up to 1,160,000 pounds per year. Half of this figure has been used in the following calculation to determine the losses during storage, transportation, and handling:

## TABLE 5-7

### Major Polyvinylidene Chloride Processors[a]

| Company | Plant location |
|---|---|
| Barrier-coating on paper and glassine | |
| Deerfield-Reed Corporation | Clifton, NJ |
| American Bag and Paper | Philadelphia, PA |
| Consolidated Paper | Wisconsin Falls, WI |
| Crown Zellerbach | Portland, OR |
| Daniels | Rhinelander, WI |
| Diversa-Pax | St. Petersburgh, FL |
| Dixico | Dallas, TX |
| DuPont | Circleville, OH |
| Milprint | Milwaukee, WI |
| Philip Morris | Nicholasville, KY |
| Rexham | Memphis, TN |
| St. Regis | Rhinelander, WI |
| Thilmany | Kaukauna, WI |
| Chase Bag Company | Hudson Falls, NY |
| Print Pak | Atlanta, GA |
| Barrier-coating on paperboard | |
| Gordon Carton | Baltimore, MD |
| Green Bay Packaging | Green Bay, WI |
| Interstate Folding Box | Middletown, OH |
| Michigan Carton Company | Battle Creek, MI |
| Olin Kraft | W. Monroe, LA |
| Zumbril | Cincinnati, OH |
| Barrier-coating on plastic | |
| DuPont | Circleville, OH |
| Cryovac | Simpsonville, SC |
| Minnesota Mining and Manufacturing | Irvington, NJ<br>Decatur, AL |
| Allied Chemical Corporation | Pittsville, PA |
| Hercules | Covington, VA |
| Milprint | Milwaukee, WI |
| Cryovac | Simpsonville, SC |
| Bemis | New London, WI |
| American Can Company | Neenah, WI |
| Standard Packaging | Clifton, NJ |
| Sealed Air Corporation | Fairlawn, NJ |

[a] Source: Neufeld et al., 1977

## TABLE 5-8
## Manufacturers of Polyvinylidene Chloride-Coated Cellophane[a]

| Manufacturer | Share of Market | Location |
|---|---|---|
| DuPont | 40% | Richmond, VA<br>Clinton, IA<br>Tecumseh, KS |
| FMC | 35% | Fredericksburg, VA |
| Olin | 25% | Pisgah Forrest, NC<br>Covington, VA |

[a] Sources: Neufeld et al., 1977; A.D. Little, Inc., 1976

## TABLE 5-9
## Major Extruders of Polyvinylidene Chloride Film[a]

| Manufacturer | Plant Location |
|---|---|
| Cryovac | Simpsonville, SC<br>Cedar Rapids, IA<br>Camarillo, CA<br>Iowa Park, TX |
| Oscar Mayer | Madison, WI<br>Chicago, IL<br>Davenport, IA<br>Philadelphia, PA<br>Nashville, TN<br>Vernon, CA<br>Sherman, TX |
| Dow Chemical | Midland, MI |
| American Can Company | Cleveland, OH |
| Union Carbide Corporation | Centerville, IA |
| Amtech, Inc.[b] | Odenton, MO |

[a] Sources: Neufeld et al., 1977; A.D. Little, Inc., 1976

[b] This plant extrudes monofilament, which is used primarily as filter cloth in the chemical industry.

## TABLE 5-10

## Estimated Emissions from Polyvinylidene Chloride Processing[a]

| Process | Emission Factor (lb/100 lb Polymer Used) | Annual Vinylidene Chloride Emissions (lb/yr) |
|---|---|---|
| Coating cellophane | 0.06 | 1,560 |
| Coating plastics, paper and glassine | 0.03 | 16,380 |
| Extrusion | 0.001 | 430 |
| Miscellaneous coating | 0.04 | 12,000 |
| TOTAL | | 30,370 |

[a] Source: A.D. Little, Inc., 1976

## TABLE 5-11

## Summary of Estimated Environmental Losses of Vinylidene Chloride

| Source | Vinylidene Chloride Contamination (lb/yr) | | |
|---|---|---|---|
| | Air | Water | Soil |
| Monomer synthesis | 446,000 | 0 | 0 |
| Polymer synthesis | 679,000 | 4100 | 0 |
| Polymer fabrication | 30,400 | 0 | 0 |
| Storage, handling, and transportation | 145,000 | 0[a] | 0[a] |
| Chemical intermediate production | 0 | 0 | 0 |
| Disposal of polymers | 0[b] | 0 | 180 |
| TOTAL | 1,300,400 | 4100 | 180 |

[a] Some water and soil contamination may arise from this source.

[b] Incineration of polymers may contaminate air with an undeterminable amount.

$$1,160,000 \times 0.5 \times 25\% = x$$

$$x = 145,000 \text{ lb/yr}$$

This annual loss of 145,000 pounds during storage, handling, and transportation does not include any additional losses due to accidental spillage during transportation.

### 5.1.5 Chemical Intermediate Production

The primary chemical intermediate that is manufactured from vinylidene chloride is chloroacetyl chloride. Since PPG Industries, the sole manufacturer of 1,1,1-trichloroethane from vinylidene chloride, no longer uses this process for 1,1,1-trichloroethane manufacture, no vinylidene chloride emissions can be expected from this intermediate. Chloroacetyl chloride is captively used by Dow Chemical Company and no losses of vinylidene chloride to the environment are reported during its manufacture (Neufeld et al., 1977). The purified chloroacetyl chloride used as an intermediate for further chemical reaction is reported to be free of vinylidene chloride contamination (Neufeld et al., 1977). Thus, its usage cannot be a source of vinylidene chloride in air.

### 5.1.6 Incineration of Polymers Containing Polyvinylidene Chloride

Over 95% of vinylidene chloride polymers are used for packaging, in the manufacture of textile fibers, or as a flame-retardant carpet backing (Neufeld et al., 1977). All these consumable items are disposed of at the end of their useful life. The ultimate disposal operation is either incineration or placement in a solid waste landfill. If incineration is used as the disposal process, it may contribute to small amounts of vinylidene chloride in air. The residual monomer in the polymer may be released into the air during heating. The decomposition of polymer to monomer is not likely to occur. The products of thermal degradation will depend primarily on the heating temperature. Thermal degradation of polyvinylidene chloride has been studied in detail by Wessling and

Edwards (1970). These investigators found that polyvinylidene chloride begins to decompose at about 125°C. At very high temperatures, graphitization of carbonaceous residue takes place. On pyrolysis, polyvinylidene chloride yields 70% hydrogen chloride, 26% carbonaceous ash, and 4% volatile hydrocarbons consisting of benzene, chlorobenzene, and three dichlorobenzenes (O'Mara et al., 1973). The thermal decomposition is also catalyzed by various metal salts. The mechanism of thermal decomposition of polyvinylidene chloride was also studied by Ballistreri et al. (1981). Currently an estimate of the amount of vinylidene chloride released to the air from incineration is not available. However, incineration is not expected to be a significant source of vinylidene chloride in air.

## 5.2 SOURCES IN WATER

The only industrial operation that produces liquid effluents containing vinylidene chloride monomer is reported to be the polymerization operation (Neufeld et al., 1977). The two main sources of vinylidene chloride loss during polymerization operations are waste polymer sludges arising from reactor cleaning during normal operation or from products containing bad batches, and wastewater from condensation of gases during stripping operations. Neufeld et al. (1977) estimate that approximately 4100 pounds per year of vinylidene chloride loss can be attributed to this source.

A small but unknown amount of vinylidene chloride may originate in water from the cleaning of rugs backed with speciality latexes.

## 5.3 SOURCES IN SOIL

The disposal of consumer products that contain processed vinylidene chloride polymers (1 to 3 ppm residual vinylidene chloride monomer) by municipal solid waste disposal operations may result in soil contamination. Neufeld et al. (1977) estimate that the maximum potential quantity of vinylidene chloride monomer in solid wastes would be 180 pounds per year.

## 5.4 SOURCES IN FOODS

The three potential sources of vinylidene chloride contamination in foods are as follows: (1) migration of monomer from the polymeric wrapping materials to foods; (2) contaminated drinking water; and (3) edible aquatic foods that have bioconcentrated vinylidene chloride from polluted water. All these are secondary sources, however, whose primary sources (i.e., polymers and water) have already been discussed.

## 5.5 SUMMARY OF ENVIRONMENTAL LOSSES

This section presents estimates of the amount of vinylidene chloride monomer lost to the atmosphere, to water streams, or to soils. The losses are summarized in Table 5-11.

# 6. ENVIRONMENTAL FATE, TRANSPORT, AND DISTRIBUTION

## 6.1 ATMOSPHERIC FATE, TRANSPORT, AND DISTRIBUTION

### 6.1.1 Reaction with Atmospheric Radicals and Ozone

Very little information is available on this subject. Upon entering the atmosphere, vinylidene chloride, like other chloro-olefin vapors, may interact with O•, OH•, and $RO_2$• radicals, and with ozone already present in the atmosphere. In addition, chloro-olefins in the atmosphere will generate chlorine atoms, which may interact with the parent molecules. The gas phase oxidation of halo-olefins at room temperature initiated by Cl atoms, $O[^3P]$ radicals, and $O_3$ has been reviewed (Sanhueza et al., 1976; Heicklen et al., 1975). In the presence of $O_2$, the oxidation of vinylidene chloride by chlorine atoms produced 98% chloroacetyl chloride, and about 2% phosgene and CO as the reaction products. The reaction of vinylidene chloride with $O[^3P]$ in the presence of $O_2$ produced CO, chloroacetyl chloride, polymer, and another unidentified compound. The rate of $O[^3P]$ attack on vinylidene chloride is the same as the rate of $O[^3P]$ attack on $C_2H_4$ (Sanhueza, 1976; Heicklen et al., 1975); however, the relative reactivity of $O[^3P]$ with vinylidene chloride (1.0) in the gas phase at room temperature is higher than the reactivity of Cl atoms with vinylidene chloride (0.72) under the same conditions (Heicklen et al., 1975). Based on a reaction rate constant of $7.8 \times 10^{-13}$ $cm^3$ $molecule^{-1}$ $sec^{-1}$ (Graedel, 1978) for $O[^3P]$ reaction with vinylidene chloride (same as $C_2H_4$ reaction rate) and the concentration of $O[^3P]$ in the ambient atmosphere as $5 \times 10^4$ molecules $cm^{-3}$ (Cupitt, 1980), the half-life for this reaction can be estimated to be approximately 206 days.

In the absence of $O_2$, $O[^3P]$ forms the same reaction products it produces in the presence of $O_2$; however, the quantum yield for carbon monoxide formation is reduced from 0.78 in the presence of $O_2$ to 0.35 in the absence of $O_2$ (Heicklen et al., 1975).

The main products of $O_3$ interaction with vinylidene chloride in the vapor phase at 25°C are phosgene and formaldehyde (Sanhueza et al., 1976; Heicklen et al., 1975; Brown et al., 1975). The formation of chloroacetyl chloride, CO, $CO_2$, HCl, and possibly water has also been reported (Hull et al., 1973). The yield of phosgene is always either comparable to or greater than the yield of formic acid (Heicklen et al., 1975). The $O_3$ reaction in the presence of $O_2$ is, however, too slow to be important in the urban atmosphere (Sanhueza et al., 1976; Heicklen et al., 1975). Based on a reaction rate constant of $4 \times 10^{-20}$ $cm^3$ $molecule^{-1}$ $sec^{-1}$ (Cupitt, 1980) and the concentration of ozone in the ambient atmosphere at $1 \times 10^{12}$ molecule $cm^{-3}$ (Cupitt, 1980), the half-life for ozone reaction with vinylidene chloride in the atmosphere can be calculated to be approximately 201 days.

The interaction of $RO_2$ and OH radicals with vinylidene chloride was reported by Brown et al. (1975). The formation of phosgene and formaldehyde was reported with both $RO_2\cdot$ and $\cdot OH$ radicals. The rate constant for the oxidation with OH radicals was reported to be $4 \times 10^{-12}$ $cm^3$ $molecule^{-1}$ $sec^{-1}$ (Cupitt, 1980). If the concentration of OH radicals in the ambient atmosphere is assumed to be $10^{-6}$ radicals $cm^{-3}$ (Cupitt, 1980), the half-life for this reaction can be calculated to be 2 days. The half-life for the peroxy radical's reaction with vinylidene chloride in the atmosphere has been reported to be about 22 years by Brown et al. (1975).

It can be concluded from the above discussion that the most significant oxidation reaction of vinylidene chloride in the ambient atmosphere is its reaction with hydroxyl radicals. The half-life for this reaction has been estimated to be about 2 days.

### 6.1.2 Atmospheric Photochemical Reactions

Gay et al. (1976) studied the photoreaction of vinylidene chloride in air in the presence of nitrogen dioxide with ultraviolet radiation. At a concentration of 4.85 ppm and in the presence of 2.26 ppm $NO_2$, vinylidene chloride was found to decompose rapidly--83% decomposition had taken place within 140 minutes. The reaction products identified were HCOOH, HCl, CO, HCHO, $O_3$, $COCl_2$, chloroacetyl chloride, formyl chloride, and nitric acid.

The photolysis of vinylidene chloride under simulated atmospheric conditions was also studied by Dilling et al. (1976). In the presence of 5 ppm NO and at a relative humidity of 35%, 10 ppm vinylidene chloride underwent photolysis at wavelengths greater than 290 nm, and disappeared with a half-life of 2.1 hours. Addition of cyclohexane was found to retard the photodecomposition rate of vinylidene chloride. Although vinylidene chloride was not tested, reaction of a closely related halo-olefin (trichloroethene) showed similar reactivity with ozone and $NO_2$ as with NO.

The above experiment of Dilling et al. (1976) was conducted at an ultraviolet radiation intensity of about 2.6 times that of natural sunlight at noon on a summer day in Freeport, TX. Therefore, the estimated half-life of vinylidene chloride under bright sunlight and in the presence of NO is approximately 5 to 12 hours (Dilling et al., 1976). In the absence of NO, the half-life for photodecomposition of vinylidene chloride under bright sunlight may increase five-fold. This estimate is based on the experimental half-life of a closely-related halo-olefin (trichloroethene) in the presence and absence of NO as determined by Dilling et al. (1976). Therefore, it is estimated that vinylidene chloride will photodecompose under bright sunlight with a half-life of one to three days. Pearson and McConnell (1975), on the other hand, exposed quartz flasks containing halogenated aliphatic compounds in air to incident radiation from the sun and estimated the tropospheric photolytic half-life to be 56 days for vinylidene chloride. Although the experiments of Pearson and McConnell (1975) were not adequately controlled in terms of air composition, temperature, and radiation intensity, it is difficult to explain the large discrepancy in the photolytic half-life of vinylidene chloride between this investigation and the work of Dilling et al. (1976). That the photolysis of vinylidene chloride in the atmosphere is a possibility has also been indicated (but no quantitative rate data given) by Cupitt (1980). In conclusion, it can be predicted that photolysis of vinylidene chloride in the atmosphere may be competitive with its reaction involving hydroxyl radicals. The half-life of vinylidene chloride in air under atmospheric smog conditions (air containing $NO_x$, $O_3$, etc.) may be further reduced due to its greater photochemical reactivity in the presence of $NO_x$ and $O_3$.

### 6.1.3 Atmospheric Physical Processes

Recently, the atmospheric fate of vinylidene chloride under physical and chemical removal processes was studied by Cupitt (1980). The atmospheric half-life of vinylidene chloride removal by rain droplets was estimated to be 110,000 years. The lifetime of vinylidene chloride removal by adsorption onto aerosol particles was estimated to be $5.3 \times 10^{10}$ days. Therefore, the possibility of vinylidene chloride removal by these two physical removal processes is remote.

## 6.2 AQUATIC FATE, TRANSPORT, AND BIOACCUMULATION

Very little experimental data pertaining to the various fate processes of this chemical in aquatic media and its bioaccumulation potential in aquatic organisms are available in the literature. In the following discussion the experimental data, whenever available, have been used to predict the fate of vinylidene chloride in aquatic media. In the absence of such data, the prediction of the fate has been based on calculated or estimated data using empirical or theoretical methods. Extreme caution should be exercised in interpreting the fate of a chemical based solely on calculated or estimated data. This reservation concerning the estimated data is justified by the large discrepancies observed in a few instances between the experimental and estimated data where both types of data are available.

### 6.2.1 Fate and Transport in Water

The photolysis and hydrolysis of vinylidene chloride in natural aquatic media are probably not significant processes (Mabey et al., 1981). No experimental data on the possibility of oxidation of vinylidene chloride by singlet oxygen ($^1O_2$) and peroxy radicals present in aquatic media could be found. However, based on the experimental data from closely related analogues (e.g., trichloroethene and tetrachloroethene), Callahan et al. (1979) concluded that vinylidene chloride is "more amenable" to oxidation in aquatic media than both trichloroethene and tetrachloroethene. From the calculated (based on structure/activity relationship) rate constant values of less than $10^8$ $M^{-1}$ $hr^{-1}$ and 3 $M^{-1}$ $hr^{-1}$ (Mabey et al., 1981) for singlet oxygen and peroxy radical reactions, respectively, with vinylidene chloride in aquatic media and the estimated concentrations of $10^{-12}$ M and $10^{-9}$ M for singlet oxygen and peroxy radicals, respectively, in aquatic media (Mill and Mabey, 1980), the oxidation of vinylidene chloride by either process can be calculated to be environmentally insignificant.

The biodegradability of vinylidene chloride was studied by Tabak et al. (1981) by the static-culture flask-screening procedure with settled domestic wastewater as microbial inoculum. These authors reported a significant degradation of vinylidene chloride with the unacclimated sludge. At 5 mg/l initial concentration of vinylidene chloride, 78% of the compound biodegraded in 7 days of incubation at 25°C. The biodegradation was only 45% when the initial vinylidene chloride concentration was 10 mg/l and the other conditions were the same. Similarly, it has been reported by Patterson and Kodukala (1981) that activated sludge treatment of a municipal wastewater at an influent vinylidene chloride level of 0.04 mg/l resulted in 97% removal of the compound. It can be concluded from these experimental results that vinylidene chloride may undergo biodegradation in ambient aquatic media. However, until the role of biodegradation of this compound in natural aquatic media is defined, it is not possible to predict the significance of this process in determining its fate in aquatic media. It should be mentioned that on the basis of limited experimental evidence on related chloroaliphatics, Callahan et al. (1979) have concluded that vinylidene chloride probably biodegrades in aquatic media at a slow rate.

The transport of vinylidene chloride from aquatic media to the atmosphere through volatilization appears to be its primary transport process (Callahan et al., 1979). The evaporation characteristics of vinylidene chloride in water were studied by Dilling (1977). When a 1 mg/l aqueous solution of 6.5 cm depth was stirred at 200 rpm in still air (<0.2 mph air current) at 25°C, the half-life for evaporation was found to be 27.2 minutes. The measured half-life for evaporation of vinylidene chloride from dilute aqueous solution was reasonably close to the calculated value of 20.1 minutes (Dilling, 1977) based on liquid and vapor-phase mass transfer coefficients for exchange between the water body and the atmosphere. However, this agreement is somewhat fortuitous since the calculated value depends on the exchange constants, whereas the experimental values depend on the stirring rate, air current, and other factors (Dilling, 1977).

The half-life for the evaporation of vinylidene chloride from pond, river, and lake waters can also be calculated from the reaeration rate constant of this compound. Assuming the reaeration rate ratio ($K_v^c/K_v^o$) of 0.601 (calculated from diffusion coefficient) (Mabey et al., 1981) for vinylidene chloride, and the values for oxygen reaeration rate constants of 0.19 $\text{day}^{-1}$, 0.96 $\text{day}^{-1}$, and 0.24

$\text{day}^{-1}$ for pond, river, and lake waters, respectively (Mabey et al., 1981), the half-lives for evaporation can be calculated to be 6.1 days, 1.2 days, and 4.2 days from pond, river, and lake waters, respectively.

No specific information pertaining to the sorption of vinylidene chloride onto sediments could be found. However, based on the experimental data from related analogues (e.g., trichloroethene and tetrachloroethene), Callahan et al. (1979) concluded that the sorption of vinylidene chloride onto sediments is probably an insignificant process. The significance of sorption of this compound onto sediments can also be predicted from the correlation equation of Schwarzenbach and Westall (1981). Assuming the octanol/water partition coefficient value of 69 for vinylidene chloride (Mabey et al., 1981), the log $K_{oc}$ value ($K_{oc} = \frac{\text{sorption coefficent} \times 100}{\%\ \text{organic carbon}}$) in sediments containing 0.5% organic carbon can be calculated to be 1.81 from the equation of Scharzenbach and Westall (1981). This log $K_{oc}$ value is somewhat lower than the experimental log $K_{oc}$ value of 1.92 for benzene (Schwarzenbach and Westall, 1981). Therefore, the rate of sorption of vinylidene chloride onto sediments is expected to be less than benzene sorption onto sediments. In other words, the sorption of vinylidene chloride onto sediments containing low organic carbon is not likely to be significant but may increase as the organic carbon content in the sediment increases.

It can be concluded from the above discussions that volatilization from aquatic media is probably the most significant transport process for vinylidene chloride. The role of biodegration in determining the fate of this compound in natural aquatic media has not been definitely determined. All other aquatic processes are probably insignificant in determining the fate of vinylidene chloride in natural aquatic media.

### 6.2.2 Bioaccumulation of Vinylidene Chloride in Aquatic Organisms

No experimental data are available for the bioaccumulation factor (BCF) for vinylidene chloride in any kind of fish or other edible aquatic organisms. A number of theoretical correlation equations are available that relate the BCF to either the octanol-water partition coefficient or the water solubility of the chemical. These equations are given below:

$\text{Log BCF} = 0.76 \log K_{ow} - 0.23$ (Veith et al., 1979)

$\text{Log BCF} = 0.542 \log K_{ow} + 0.124$ (Neely et al., 1974)

$\text{Log BCF} = -0.508 \log S + 3.41$ (Chiou et al., 1977)

where $K_{ow}$ = partition coefficient of the chemical between octanol/water, and

$S$ = water solubility of the chemical expressed in μmol/l.

The above equation of Veith et al. (1979) is applicable for the whole fish, whereas the equations of Neely et al. (1974) and Chiou et al. (1977) are applicable for fish muscle only.

If the values of log $K_{ow}$ and S for vinylidene chloride are assumed to be 1.48 (Tute, 1971) and $2.19 \times 10^3$ μmol/l (Hushon and Kornreich, 1978), respectively, the theoretical values for BCF can be calculated to be 7.8 (equation of Veith et al., 1979) for the whole fish and 8.5 (equation of Neely et al., 1974) or 51 (equation of Chiou et al., 1977) for fish muscle. The relatively low value for BCF indicates that vinylidene chloride will probably not bioaccumulate in fish to any significant extent.

## 6.3 FATE, PERSISTENCE, AND TRANSPORT IN SOIL

No information pertaining specifically to the fate, persistence, and transport of vinylidene chloride in soils could be found. However, the fate and transport processes of this compound can be partially predicted from the estimated log $K_{oc}$ value of 1.81 in soils containing 0.5% organic carbon. This relatively low log $K_{oc}$ value, coupled with a solubility of 2250 mg/l and a vapor pressure of 600 mm of mercury at 25°C, would indicate that both volatilization and leaching may play significant roles in determining the fate of this chemical in soils. In fact, the detection of this compound in several groundwaters (see Section 7.2) is indicative of the leaching of vinylidene chloride from soils. Unless more experimental or calculated data are available, no prediction regarding the microbial degradability and chemical oxidation of this chemical in soils can be made, although Kobayashi and Rittmann (1982) have reported the biodegradation of vinylidene chloride by soil bacteria.

# 7. ENVIRONMENTAL LEVELS AND EXPOSURE

When an environmental pollutant is suspected of being a potential health hazard, it becomes necessary to determine the level of that pollutant in the environment and its possible human intake from various environmental media. The four environmental media of interest in this regard are air, water, soil, and foods. The vinylidene chloride levels from each individual medium and the possible human exposure therefrom are discussed in the following subsections.

The purpose of this document is to present available information relevant to human health effects that could be caused by this substance. Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

## 7.1 AIR

### 7.1.1 Environmental Levels

The concentration of vinylidene chloride in air can be determined by monitoring its level in the atmosphere. In the absence of monitored data, the concentration can be theoretically estimated by dispersion modelling based on the amount of vinylidene chloride losses in air from each plant. Such theoretical modelling, which is generally recognized to be accurate within a factor of two in predicting the concentration of a pollutant in the vicinity of a plant, has been used for estimating the population exposure to other atmospheric pollutants, such as acrylonitrile (Suta, 1979). A vinylidene chloride model based on analogy to 1,1,1-trichloroethane has been developed to estimate potential human exposure (Wapora, 1982). Using dispersion modelling, Hushon and Kornreich (1978) estimated the vinylidene chloride concentration levels at a single distance from three vinylidene chloride monomer production sites in the United States using atmospheric dispersion modeling. The 10-minute peak exposures of an individual 500 m downwind from the three plants were estimated to be 0.87 ppm, 0.44 ppm, and 0.52 ppm for the Dow plant at Freeport, the Dow plant at Plaquemine, and the PPG plant at Lake Charles, respectively. The corresponding 24-hour peak concentrations at the same three locations were estimated to be 0.52 ppm, 0.26 ppm, and 0.32 ppm. In order to estimate these values, it was assumed that these plants have certain yearly emission rates of vinylidene chloride (Hushon and Kornreich, 1978), the wind speed was 6 m/s, the height of the plants was 12.3 m, and vinylidene chloride monomer would be non-reactive in the atmosphere. The last assumption may have led to erroneous estimates of vinylidene chloride concentration at longer distances. The half-life of vinylidene chloride under simulated smog conditions was estimated to be 5 to 12 hours (Dilling et al., 1976) and approximately 2 days under atomspheric hydroxyl reactions (Cupitt, 1980). This relatively rapid atmospheric oxidation may not have a significant effect on ambient air concentration at short distances from the source, but may have a dramatic effect at longer distances. Thus, any dispersion modelling for estimation of vinylidene chloride concentrations at various distances from the source should include the effect of disappearance of vinylidene chloride due to atmospheric oxidation.

One of the earlier attempts to experimentally determine atmospheric levels of halocarbons including vinylidene chloride was made by Grimsrud and Rasmussen (1975) in rural Pullman, WA, during the period of December 1974 to February 1975. They used a GC-MS method for the quantification of vinylidene chloride. Because of the limitations of the analytical methodology (inadequate GC column), however, they could not separate vinylidene chloride from its isomers cis- and trans-1,2-dichloroethylene, and reported the combined concentration of the three isomers to be <5 ppt.

The monitoring of vinylidene chloride near industrial sites was conducted by Research Triangle Institute (Research Triangle Institute, 1977a,b, cited in Hushon and Kornreich, 1978) during the period 1976 to 1977. Vinylidene chloride concentrations ranging from 9 ppt to 249 ppt were detected near Dow plant sites, one at Freeport, TX, and four at Plaquemine, LA. Seven other Dow sites at Plaquemine, LA, showed no detectable trace of vinylidene chloride.

The more systematic environmental monitoring for vinylidene chloride near industrial sites was performed by Going and Spigarelli (1977) during the period of January 1977 to April 1977. These investigators selected sampling points near six industrial plants representing manufacturers of vinylidene chloride monomers and polymers and fabricators of the polymers. The analyses of the samples were performed by GC-FID and GC-MS.

The concentrations of vinylidene chloride in the ambient air throughout the U.S. have been measured by several other investigators (Bozzelli et al., 1980; Going and Spigarelli, 1977; Pellizzari et al., 1978a, b; Pellizzari et al., 1979; Pellizzari and Bunch, 1979; Wallace et al., 1984). Based on an assessment of quality as evaluated by the reliability of sampling and analytical methodology and the quality control/quality assurance strategy employed in the measurements (Brodzinsky and Singh, 1982), the monitoring data from these studies that are considered acceptable are presented in Table 7-1. In the studies with the source areas, the durations of sampling were approximately twenty-four hours. Concentrations of vinylidene chloride in air samples collected in 1979 from Los Angeles, CA; Phoenix, AZ; and Oakland, CA, were measured by Singh et al. (1981b). The mean concentrations were determined to be 4.9, 29.8, and 12.6 ppt, respectively. However, these values have not been presented in Table 7-1 because no assessment of the quality of these data is available.

The sampling sites, sampling data, total number of sampling points monitored at each site, the mean concentrations, the standard deviations, the median concentrations, and the concentration ranges at each site are shown in Table 7-1. Additionally, the corresponding values for the eight sites combined, and the values for the sampling points categorized as urban/suburban and as source areas are also shown in Table 7-1. It is obvious from Table 7-1 that the median concentration of vinylidene chloride in U.S. urban/suburban ambient air is approximately 5 ppt. However, the concentration is substantially higher (2182 ppt) for ambient air samples collected from vinylidene chloride source areas.

### 7.1.2 Exposure

The Office of Air Quality Planning and Standard (OAQPS), U.S. EPA, Research Triangle Park, NC, has prepared a document estimating human exposure to vinylidene chloride in the atmosphere around plants producing the monomer and the polymer. The ranges of measured concentrations associated with the monomer and polymer plants were 90-100 $\mu g/m^3$ and 25-50 $\mu g/m^3$, respectively. For a detailed discussion, the readers are referred to the OAQPS document (Wapora, 1982).

Landau and Manos (1976) estimated the number of Americans who reside within 5 miles of 38 major plants in the United States that produce monomers and

## TABLE 7-1

The Mean, Median, and Range of Vinylidene Chloride Concentrations at Different Sites in the U.S.

| Location | Sampling Date | Number of Sampling Points | Mean Conc. (ppt) | Std.[a] Dev. (ppt) | Median Conc. (ppt) | Minimum Conc. (ppt) | Maximum Conc. (ppt) | Reference |
|---|---|---|---|---|---|---|---|---|
| Denver, CO | 1980 | 85 | 6.4 | 21 | 1.4 | ND | 135.8 | Singh et al., 1981a |
| Houston, TX | 1977 | 75 | 16.0 | 15 | 13.7 | 2.6 | 55.0 | Singh et al., 1981a |
| Riverside, CA | 1980 | 87 | 6.5 | 3.0 | 6.5 | 1.4 | 13.9 | Singh et al., 1981a |
| S. Amboy, NJ | 1979 | 1 | ND | 0 | ND | ND | ND | Bozzelli et al., 1980 |
| St. Louis, MO | 1980 | 78 | 3.6 | 1.7 | 3.6 | ND | 6.5 | Singh et al., 1981a |
| Lake Charles, LA[b] | 1977 | 5 | 6660.0 | 73.0 | 5752 | 1236 | 13068.0 | Going and Spigarelli, 1977 |
| Midland, MI[b] | 1977 | 2 | 6054.0 | 6.0 | 6054.0 | 5802.0 | 6307.0 | Going and Spigarelli, 1977 |
| Plaquemine, LA[b] | 1977 | 7 | 1138.0 | 69.0 | 959.0 | 151.0 | 2447.0 | Going and Spigarelli, 1977 |
| Total Sites | | 340 | 164.6 | 93.0 | 5.0 | ND | 6659.0 | |
| Urban/Suburban Sites | | 326 | 7.9 | 13.7 | 5.0 | ND | 135.8 | |
| Source Areas | | 14 | 3813.0 | 2780.0 | 2182.0 | 151.0 | 13068.0 | |

[a] Standard Deviation

[b] These are all source areas

ND = Not Detected

polymers and fabricate polymers. The estimated values are given in Table 7-2. The values are only population estimates and are not meant to indicate the absolute number of people exposed to vinylidene chloride. The size of the population residing near plant sites as given in Table 7-2 may have changed slightly due to shut down of a few plants or addition of new plants and due to some population shifts.

The estimation of the number of people exposed to vinylidene chloride would require knowledge of the chemical's transport characteristics. Theoretically, dispersion modelling could provide an estimate of vinylidene chloride concentrations at different distances from the plant sites; however, such data are not presently available.

Experimental data could provide a solution to this problem. Going and Spigarelli (1977) detected vinylidene chloride at a distance of 1.5 miles from a vinylidene chloride monomer production facility. Their study was not designed to determine vinylidene chloride levels at different distances from the source. Therefore, the study of Going and Spigarelli (1977) cannot be used to estimate vinylidene chloride exposure.

With the median ambient levels (averaged over 1 hour up to 1091 days) given in Table 7-1, and assuming 20 $m^3$ of air is inhaled per day by an individual, the estimated daily vinylidene chloride intake from urban/suburban areas through inhalation is approximately 0.4 µg. Pellizzari et al. (1979) estimated the daily average vinylidene chloride intake through inhalation of ambient air from the Baton Rouge, Geismar, and Plaquemine, LA, area and the Houston, Deer Park, and Pasadena, TX, area to be 0.7 µg and 1.6 µg, respectively. However, the daily inhalation exposure may be as high as 0.17 mg in the downwind direction near the immediate vicinity of source areas if a median vinylidene chloride level of 2182 ppt (see Table 7-1) is employed to calculate the exposure value.

## TABLE 7-2

### Estimated Population Residing Near Plant Producing or Fabricating Monomers and Polymers of Vinylidene Chloride[a]

| Perimeter (miles) | Producing or process plants | | | Total |
|---|---|---|---|---|
| | Monomer | Polymer | Fabrication | |
| 0-1/2 | 315 | 11,114 | 37,310 | 48,739 |
| 1/2-1 | 1,035 | 34,091 | 100,974 | 136,100 |
| 1-3 | 17,356 | 221,966 | 1,064,994 | 1,304,316 |
| 3-5 | 70,230 | 337,547 | 1,676,463 | 2,084,240 |
| TOTAL | 88,936 | 604,718 | 2,879,741 | 3,573,395 |

[a] Source: Landau and Manos, 1976

## 7.2 WATER

### 7.2.1 Environmental Levels

The presence of vinylidene chloride in water samples was reported as early as 1975. Monitoring investigations performed prior to or during 1976 have reported the presence of vinylidene chloride in a wide range of water samples ranging from industrial effluents to drinking waters. Some of the earlier attempts at analysis, however, could not separate vinylidene chloride from its isomers due to inadequate GC column selection. The presence of unseparated vinylidene chloride and its isomers has been reported in effluents from the textile industry, effluents from the latex industry, effluents from sewage treatment plants, raw surface waters, finished drinking waters, and even in well water (Schackelford and Keith, 1976).

The presence of vinylidene chloride alone, separated from its isomers, has been reported four times in industrial effluents, two times in raw surface waters, seven times in finished drinking water, and one time in well water (Shackelford and Keith, 1976). No quantitative values for vinylidene chloride concentrations are available from most of these earlier reports, however, because the vinylidene chloride concentrations were very low and mass spectrometry was used for its identification and not for quantification. The levels of vinylidene chloride in industrial raw wastewaters and treated wastewaters have been determined recently (U.S. EPA, 1981) and are shown in Table 7-3 and Table 7-4. These tables also indicate the expected vinylidene chloride loading in surface waters due to the discharge of the wastewaters into these water bodies. According to these tables, nonferrous metals manufacturing industries are likely to contribute to the maximum vinylidene chloride loading into the water bodies.

In a recent publication, Keith and Telliard (1979) reported the approach used by the U.S. EPA in establishing a priority pollutant list for toxic

TABLE 7-3

Industrial Occurrence of Vinylidene Chloride in Raw Wastewater[a,b]

| Industry | Number of samples | Number of detections | Concentration, μg/l Minimum | Concentration, μg/l Maximum | Concentration, μg/l Mean | Loading, kg/d[c] mean |
|---|---|---|---|---|---|---|
| Auto and other laundries[d] | 5 | 3 | 2.0 | 23 | 7.5 | 0.0014 |
| Coal mining[e] | 47 | 3 | 3 | 3 | 3 | 0.011 |
| Aluminum forming | NA | ND | | | | |
| Battery manufacturing | 27 | 8 | ND | 10 | 10 | 0.0008 |
| Coil coating | 30 | 2 | ND | 36 | 10 | 0.0011 |
| Metal finishing[e] | 95 | 16 | 2 | 10,000 | 760 | 0.61 |
| Porcelain enameling | 1 | ND | | | | |
| Nonferrous metals manufacturing | 66 | 9[f] | ND | 6,100 | 200 | 1.6 |
| Organic chemicals manufacturing/plastics | NA | 22 | NA | NA | 200 | NA |
| Paint and ink formulation[d] | 36 | 8 | NA | 620 | 78 | 0.00031 |
| Soap and detergent manufacturing[d] | 3 | 3 | 11 | 25 | 18 | 0.0016 |
| Steam electric power plants[g] | 11 | ND | | | | |
| Textile mills[e] | 50 | 1 | | <5 | | <0.0035 |

[a] Source: U.S. EPA, 1981

[b] The pollutant was not detected during screening analyses for the following industries: Leather tanning and finishing, Foundries, Explosives manufacture, Gum and wood chemicals, Pharmaceutical manufacturing, Nonferrous metals manufacturing, Pulp and paperboard mills, Rubber processing, Timber products processing.

[c] Pollutant loadings determined by multiplying mean pollutant concentration by industry wastewater discharges; where mean is not available one-half the reported maximum was utilized.

[d] Screening data

[e] Screening and verification data

[f] Detections >10 μg/l

[g] Verification data plus surveillance and analyses

NA = Not Available; ND = Not Detected

## TABLE 7-4

### Industrial Occurrence of Vinylidene Chloride in Treated Wastewater[a,b]

| Industry | Number of samples | Number of detections | Concentration, µg/l |  |  | Loading, kg/d[c] mean |
|---|---|---|---|---|---|---|
|  |  |  | Minimum | Maximum | Mean |  |
| Auto and other laundries[d] | 1 | ND |  |  |  |  |
| Coal mining[e] | 51 | 3 | 3 | 3 | 3 | 0.0056 |
| Coil coating | 5 | ND |  |  |  |  |
| Explosives manufacture | NA | NA | NA | NA | NA | NA |
| Pharmaceutical manufacturing | 2 | 2 | 10 | 10 | 10 | 0.0091 |
| Nonferrous metals manufacturing | 70 | 10[f] | ND | 4,100 | 120 | 0.96 |
| Organic chemicals manufacturing/ plastics | NA | 15 | NA | NA | 6.8 | NA |
| Paint and ink formulations[d] | 24 | 4 | NA | 44 | 19 | 0.000076 |
| Soap and detergent manufacturing | NA | NA | NA | NA | NA | NA |
| Steam electric power plants[g] | 12 | 1 |  | 10 |  | 0.015 |

[a] Source: U.S. EPA, 1981

[b] The pollutant was not detected during screening analyses for the following industries: Leather Tanning and Finishing, Aluminum Forming, Battery Manufacturing, Foundries, Porcelain Enameling, Gum and Wood Chemicals, Rubber Processing, Timber Products Processing.

[c] Pollutant loading determined by multiplying mean pollutant concentration by industry wastewater discharges; where mean is not available, one-half the reported maximum was utilized.

[d] Screening data

[e] Screening and verification data

[f] Detections >10 µg/l

[g] Verification data plus surveillance and analyses

NA = Not Available; ND = Not Detected

chemicals. Industrial effluents were screened by GC-MS for various pollutants, including vinylidene chloride. Among the over 2,000 samples representing 17 different industrial categories that were analyzed up until August 1978, 7.7% qualitatively showed the presence of vinylidene chloride.

The levels of vinylidene chloride in surface waters around a few industrial sites were determined by Going and Spigarelli (1977). The samples were analyzed by a combination of GC-FID and GC-MS. Table 7-5 lists the concentrations of vinylidene chloride in these water samples.

The levels of vinylidene chloride in U.S. raw surface and ground waters used for the abstraction of drinking waters and in potable drinking waters were monitored as part of the National Organic Monitoring Survey (NOMS) (U.S. EPA, 1977) and National Organics Reconnaissance Survey (NORS) (Coleman et al., 1976). The results of these two surveys were combined with four other surveys conducted for the U.S. EPA (namely, surveys by Stanford Research Institute, Office of Toxic Substances (OTS), Annapolis Field Study and U.S. EPA Region V Survey) by Coniglio et al. (1980) to derive from statistics the levels of volatile organics in raw and finished waters in the U.S. None of the surface waters from the 105 cities monitored in this survey showed any detectable level of vinylidene chloride. Only two finished waters abstracted from surface waters from 103 cities showed the presence of this compound at concentrations of 0.2 μg/l and 0.51 μg/l. Since the compound was not present in the raw water, it is likely that the treatment process is the source of vinylidene chloride in these treated waters. Going and Spigarelli (1977) also monitored the treated surface waters from Cincinnati, OH, Philadelphia, PA, and Lawrence, MA, for vinylidene chloride. Neither the pre-chlorinated nor the post-chlorinated treated water from any of these cities showed any detectable level of vinylidene chloride. Recently, Pellizzari et al. (1979) reported the mean concentration of vinylidene chloride in the drinking water from New Orleans and Baton Rouge, LA, areas to be 0.2 μg/l.

TABLE 7-5

Vinylidene Chloride Concentration in a Few Waters Near Industrial Sites[a]

| Sample source[b] | Industrial site | Industrial process | Frequency of detection[c] | Concentration High (μg/l) | Concentration Low (μg/l) |
|---|---|---|---|---|---|
| Mississippi River | Dow Chemical, Plaquemine, LA | Monomer producer | 1/2 | 0.2 | ND |
| PPG Canal | PPG Industries Lake Charles, LA | Monomer and methyl chloroform producer[d] | 1/2 | 550 | ND |
| Ohio River | W.R. Grace, Owensboro, KY | Polymer producer | 0/2 | ND | ND |
| Holston River | Eastman, Kingsport, TN | Modacrylic producer | 2/4 | 1 | ND |
| Tittabawasse River | Dow Chemical, Midland, MI | Polymer producer and extruder | 1/3 | <1 | ND |

[a] Source: Going and Spigarelli, 1977

[b] The samples were collected at different points upstream and downstream from the discharge outfall.

[c] The first figure indicates the number of samples that showed presence of compound and the second figure indicates the total number of samples analyzed.

[d] This plant no longer uses vinylidene chloride to produce methyl chloroform.

ND = Not Detected.

A survey of raw and treated groundwaters from 13 U.S. cities was also conducted by Coniglio et al. (1980). Raw groundwaters from two cities both showed detectable quantities of vinylidene chloride at a level of 0.5 μg/l. Vinylidene chloride was detected in treated groundwater from only one city at a concentration of 0.2 μg/l. Going and Spigarelli (1977) also detected 0.059 μg/l and 0.045 μg/l of vinylidene chloride in pre-chlorinated and post-chlorinated treated groundwater, respectively, from Miami, FL. Eight state agencies (e.g., AL, FL, KY, ME, MA, NC, SC, and TN) also monitored the levels of dichloroethylenes (three unseparated isomers) in their well water. Of the total of 781 samples tested, 23% showed detectable levels of dichloroethylenes with a maximum level of 860 μg/l (Coniglio et al., 1980). The levels of vinylidene chloride in 378 groundwater samples from urban, suburban, rural, and dump site areas in New Jersey were monitored by Page (1981). Approximately 44% of the groundwaters showed detectable levels of vinylidene chloride. Although the highest concentration of vinylidene chloride detected in a groundwater, probably from a dump site (author did not specify the site), was 17.3 mg/l, the median concentration of vinylidene chloride in all samples was below the detection limit (10 μg/l).

The levels of vinylidene chloride in raw and treated (both ground and surface waters) from 30 Canadian potable water treatment facilities serving about 5.5 million consumers across Canada were monitored by Otson et al. (1982a,b). Vinylidene chloride was not detected in any of the raw water samples, and only one finished water sample collected during the summer months (Aug.-Sept.) showed a mean concentration of less than 1 μg/l and a maximum concentration of 20 μg/l for vinylidene chloride.

### 7.2.2 Exposure

It can be concluded from the discussions in Section 7.2.1 that about 3% of the total drinking water supplies in the U.S. contain vinylidene chloride at an estimated mean concentration of 0.3 µg/l. Assuming that an individual consumes 2 liters of water per day, the daily exposure to vinylidene chloride for these consumers (3% water supplies) would be approximately 0.6 µg. For the majority of the U.S. population, the daily exposure to vinylidene chloride from the consumption of drinking water would be lower than 0.01 µg, based on a detection limit of 0.005 µg/l as determined by Going and Spizarelli (1977). Therefore, the daily exposure of vinylidene chloride to the general population in the U.S. from the consumption of drinking water would be <0.01 µg, although the maximum daily exposure in certain communities could exceed 1 µg (see Section 7.2.1).

## 7.3 SOIL

DeLeon et al. (1980) analyzed over 100 soil samples within and around a few chemical waste disposal sites for 11 chlorinated hydrocarbons including vinylidene chloride. The analytical methods used were GC-ECD for quantification and GC-MS for confirmation. One of the three samples collected from a midwestern chemical disposal site (name not mentioned) showed 21.9 µg/g (dry weight) of vinylidene chloride, while none was detected in the other two samples. The detection limit of the method was given as 10 µg/g.

## 7.4 FOODS

Human exposure to vinylidene chloride from foods can occur in two ways: from consumption of food contaminated by polyvinylidene wrappings, and from consumption of edible aquatic foods that have bioconcentrated vinylidene chloride from water. Vinylidene chloride content has been determined both in food-packaging materials and in the foods themselves.

The results of analysis of vinylidene chloride content in saran and meat packaging films as determined by Going and Spigarelli (1977) are shown in Table 7-6. Hollifield and McNeal (1978) analyzed five commercial food-packaging saran films of 0.5 mil thickness and determined that the residual monomer concentration varied between 1.6 µg/g and 8.1 µg/g.

## TABLE 7-6

## Analysis of Food-Packaging Films for Vinylidene Chloride[a]

| Sample | Concentration (µg/g) |
|---|---|
| Saran Wrap | 41-58[b] |
| Saran Wrap | 4.9 |
| Oscar Mayer Film A | ND |
| Oscar Mayer Film B | ND |
| Oscar Mayer Film C | ND |

[a] Source: Going and Spigarelli, 1977

[b] This saran wrap, presumably produced in 1974, contained higher amounts of vinylidene chloride.

ND = Not Detected. The detection limit was 0.005 µg/g.

The extent of migration of residual vinylidene chloride from polyvinylidene packaging materials into foods is customarily tested with food-simulating solvents rather than with foods themselves (Hollifield and McNeal, 1978). The three food-simulating solvents, which represented aqueous, oily, and fatty foods, were water, corn oil, and heptane, respectively (Hollifield and McNeal, 1978). The extent of migration of vinylidene chloride from saran films to food-simulating solvents at 49°C is shown in Table 7-7.

It can be concluded from Table 7-7 that the migration of the residual monomer from the 0.5 mil film was essentially complete in corn oil and heptane in well under 24 hours. It leveled off at about 60% in water after 24 hours.

It is difficult to estimate the extent of human exposure to vinylidene chloride resulting from consumption of food contaminated by polyvinylidene packaging materials. The exposure to vinylidene chloride from consuming contaminated edible aquatic foods is equally difficult to estimate, even when the bioaccumulation factor is available. To calculate body burden, a knowledge of the concentration of vinylidene chloride in the water from which the aquatic organism originated is necessary.

## TABLE 7-7

### Migration of Vinylidene Chloride from Saran Films to Heptane, Corn Oil, and Water at 49°C[a]

| Film Thickness | Residue monomer concentration µg/g | Time of contact (hours) | Concentration found in solvent (ppb) | Percent of total extracted |
|---|---|---|---|---|
| Heptane | | | | |
| 0.5 mil | 8.1 | 0.5 | 34 | 76% |
| | | 1.0 | 37 | 88% |
| | | 2.0 | 41 | 94% |
| | | 2.5 | 44 | 106% |
| Corn Oil | | | | |
| 0.5 mil | 8.1 | 1 | 18 | 41% |
| | | 3 | 34 | 76% |
| | | 6 | 40 | 94% |
| | | 24 | 39 | 94% |
| | | 48 | 41 | 94% |
| Water | | | | |
| 0.5 mil | 8.1 | 4 | 26 | 59% |
| | | 24 | 27 | 65% |
| | | 312 | 24 | 59% |
| | | 696 | 24 | 59% |

[a] Source: Hollifield and McNeal, 1978

## 8. BIOLOGICAL EFFECTS ON PLANTS AND MICROORGANISMS

The 96-hour $EC_{50}$ (median effective concentration) values for the freshwater alga *Selenastrum capricornutum* and the marine alga *Skeletonema costatum* were found to be greater than 798 or 712 mg vinylidene chloride/l, respectively (U.S. EPA, 1978). These were the highest concentrations tested. The reported "no effect concentration" was <80 mg/l for *Selenastrum capricornutum* and 712 mg/l for *Skeletonema costatum*. The criteria for toxic effect in these bioassays were reduction in cell number or chlorophyll *a* content in algae cultures grown for 96 hours.

No studies were found in the literature searched on the biological effects of vinylidene chloride on microorganisms.

# 9. BIOLOGICAL EFFECTS ON AQUATIC ORGANISMS

## 9.1 ACUTE TOXICITY

The acute toxicity of vinylidene chloride has been determined with several species of freshwater and marine fish and invertebrates. Acute toxicity values are presented in Table 9-1.

### 9.1.1 Freshwater Fish

The 96-hour $LC_{50}$ (95% confidence interval) for bluegill sunfish, Lepomis macrochirus, tested under static exposure conditions was 73.9 (56.8-90.7) mg/l (U.S. EPA, 1978; Buccafusco et al., 1981). This value is the same as the 24-hour $LC_{50}$ (Table 9-1), indicating that all mortalities during the test occurred within the first day of exposure. The "no effect" concentration was 32.0 mg/l. Dawson et al. (1977) reported a much higher 96-hour $LC_{50}$ for bluegill sunfish (220 mg/l). The chemical used was identified as "vinylidene chlorine" and presumably was vinylidene chloride. This test was conducted under static exposure conditions. Dill et al. (n.d., cited in U.S. EPA, 1979) determined the 96-hour $LC_{50}$ for fathead minnows, Pimephales promelas, under static and flow-through exposure conditions. The values were 169 mg/l and 108 mg/l, respectively. The different results indicate that the flow-through technique gave a higher estimate of the acute toxicity of vinylidene chloride than did the static exposure technique.

### 9.1.2 Freshwater Invertebrates

The water flea, Daphnia magna, is the only freshwater invertebrate for which vinylidene chloride toxicity data are available. The 24-hour and 48-hour static $LC_{50}$ (95% confidence interval) values were 98 (71-130) and 79 (62-110) mg/l, respectively (LeBlanc, 1980). The "no discernible effect" concentration was <2.4 mg/l, which was the lowest concentration tested. In another study (Dill

TABLE 9-1

Acute Toxicity Values for Marine and Freshwater Fish and Invertebrates Exposed to Vinylidene Chloride

| Species | Test Type | Water Type | LC50 (mg/1)[a] | | | | No Effect Concentration (mg/1) | References |
|---|---|---|---|---|---|---|---|---|
| | | | 24-h | 48-h | 72-h | 96-h | | |
| **FISH** | | | | | | | | |
| Bluegill Sunfish (*Lepomis macrochirus*) | ST | FW | 73.9<br>(56.8-90.7) | 73.9<br>(56.8-90.7) | 73.9<br>(56.8-90.7) | 73.9 | 32.0 | U.S. EPA, 1978<br>Buccafusco et al., 1981 |
| | ST | FW | -- | -- | -- | 220 | -- | Dawson et al., 1977 |
| Fathead Minnows (*Pimephales promelas*) | ST | FW | -- | -- | -- | 169 | | Dill et al., n.d. |
| | ST | FW | -- | -- | -- | 108 | -- | Dill et al., n.d. |
| Sheepshead Minnow (*Cyprinodon variegatus*) | ST | SW | 249<br>(198-338) | 249<br>(198-338) | 249<br>(198-338) | 249<br>(198-338) | 79.8 | U.S. EPA, 1978<br>Heitmuller et al., 1981 |
| Tidewater Silversides (*Menidia beryllina*) | ST | SW | -- | -- | -- | 250 | -- | Dawson et al., 1977 |
| **INVERTEBRATES** | | | | | | | | |
| Water Fleas (*Daphnia magna*) | ST | FW | -- | 11.6[b] | -- | -- | -- | Dill et al., n.d. |
| Water Fleas (*Daphnia magna*) | ST | FW | 98<br>(71-130) | 79<br>(62-110) | -- | -- | <2.4 | LeBlanc, 1980 |
| Mysid Shrimp (*Mysidopsis bahia*) | ST | SW | >798 | >798 | >798 | 224<br>(106-434) | 14.2 | U.S. EPA, 1978 |

[a] Numbers in parentheses represent the 95% confidence interval

[b] Median effective concentration ($EC_{50}$)

ST = static exposure; FT = flow-through exposure; FW = freshwater; SW = sea water et al., n.d., cited in U.S. EPA, 1979), the 48-hour static $EC_{50}$ (median effective concentration) was 11.6 mg/l, which is much lower than the previously cited $LC_{50}$ values. The difference may be due to the different toxicity endpoints measured (effective concentration vs. lethal concentration) or some other factor(s).

### 9.1.3 Marine Fish

The 96-hour $LC_{50}$ (95% confidence interval) for sheepshead minnows, Cyprinodon variegatus, tested under static exposure conditions, was 249 (198-338) mg/l (U.S. EPA, 1978; Heitmuller et al., 1981). As occurred with bluegill sunfish, the 96-hour $LC_{50}$ was the same as the 24-hour $LC_{50}$ (Table 9-1). The "no effect" concentration was 79.8 mg/l. The only other study with marine fish was by Dawson et al. (1977), who reported a 96-hour $LC_{50}$ value of 250 mg/l for tidewater silversides, Menidia beryllina, tested under static exposure conditions. This value is nearly identical to the 96-hour $LC_{50}$ for sheepshead minnows.

### 9.1.4 Marine Invertebrates

The mysid shrimp, Mysidopsis bahia, is the only species of marine invertebrate that has been tested for vinylidene chloride toxicity. The 96-hour $LC_{50}$ value (95% confidence interval) for this species was 224 (106-434) mg/l. The $LC_{50}$ value at 24, 48, and 72 hours could not be determined precisely, but was greater than 798 mg/l for each time period. The "no effect" concentration was 14.2 mg/l. The 96-hour $LC_{50}$ value for mysid shrimp was similar to the 96-hour $LC_{50}$ values for sheepshead minnows and tidewater silversides.

## 9.2 SUBACUTE TOXICITY

The only information regarding the subacute effects of vinylidene chloride on aquatic organisms concerns one species of freshwater fish.

Dill et al. (n.d., cited in U.S. EPA, 1979) determined the 13-day $LC_{50}$ for fathead minnows under flow-through conditions. This value, 29 mg/l, was considerable lower than the equivalent 96-hour $LC_{50}$ value (108 mg/l).

No adverse effects were observed in a fathead minnow embryo-larval test at 2.8 mg/l, which was the highest concentration tested (U.S. EPA, 1978). In this test, fathead minnow eggs were exposed under flow-through conditions to a series of vinylidene chloride concentrations through the hatching and early larval life stages.

Most of the previously cited aquatic toxicity tests were conducted under static exposure conditions. As far as can be determined, the $LC_{50}$ values obtained from these tests were calculated on the basis of initial nominal concentrations, rather than actual measured concentrations.

Due to the high volatility of vinylidene chloride (Section 3), it is probable that test organisms in static toxicity tests would be exposed to rapidly decreasing concentrations of vinylidene chloride. This rapid loss may account for the observations that the static $LC_{50}$ values for bluegill sunfish and sheepshead minnow (U.S. EPA, 1978) did not decrease between 24 and 96 hours of exposure and that the static 96-hour $LC_{50}$ for fathead minnows was higher than the flow-through 96-hour $LC_{50}$ with the same species (Dill et al., cited in U.S. EPA, 1979). Although the static tests with bluegills and sheepshead minnows produced apparent lethal thresholds by 24 hours of exposure, Dill et al. (n.d., cited in U.S. EPA, 1979) showed that flow-through exposure with fathead minnows resulted in sufficient additional mortality between 4 and 13 days of exposure to lower the $LC_{50}$ from 108 to 29 mg/l. Although static tests may provide some useful information concerning the potential ecological effects of vinylidene chloride in spill situations, the flow-through results indicate that static tests may under estimate the effects of acute and chronic exposure to constant vinylidene chloride concentrations.

In summary, the reported acute $LC_{50}$ or $EC_{50}$ values for four fish species and two invertebrate species ranged between 11.6 and 250 mg/l. The acute static "no effect" concentrations reported in U.S. EPA (1978) decreased in this order: sheepshead minnow (79.8 mg/l) > bluegill sunfish (32.0 mg/l) > mysid shrimp (14.2) > Daphnia magna (<2.4 mg/l, the lowest concentration tested). However, insufficient species have been tested to definitely establish whether or not there may be significant differences in sensitivity to vinylidene chloride between marine and freshwater organisms, or between fish and invertebrates. Of the species tested, the most sensitive species appears to be Daphnia magna, which was adversely affected at 2.4 mg/l, the lowest concentration tested with that species in the U.S. EPA (1978) study. This concentration is lower than the concentration (2.8 mg/l) reported in the same study to cause no adverse effects on fathead minnows in a flow-through embryo-larval test.

Additional flow-through chronic or subchronic testing with Daphnia magna and other freshwater and marine species would be required to estimate more accurately the range of species sensitivity and to determine the maximum acceptable concentrations of vinylidene chloride compatible with protection of aquatic life.

# 10. BIOLOGICAL EFFECTS IN ANIMALS AND MAN

## 10.1 PHARMACOKINETICS

The available data characterize the disposition and metabolism of vinylidene chloride in experimental animals when administered as a single dose or exposure. The effects of age, sex, route, species and fasting on disposition and metabolism are also characterized. Information on the disposition and metabolism of vinylidene chloride during subchronic or chronic exposure, however, is lacking. It should be noted that throughout Section 10.1, animals can be assumed to be fed unless fasting is specified.

### 10.1.1 Absorption and Distribution

Studies with experimental animals indicate that vinylidene chloride is readily absorbed and rapidly distributed in the body. Systemic absorption after intragastric administration of $^{14}C$-vinylidene chloride to male or female rats appears to be rapid and fairly complete based on the rapid appearance of labeled vinylidene chloride in the expired air (McKenna et al., 1978b; Reichert et al., 1979) and the extensive pulmonary and urinary excretion of radioactivity (Jones and Hathway, 1978a; McKenna et al., 1978b; Reichert et al., 1979). Intraperitoneal administration also resulted in extensive pulmonary and urinary excretion (Jones and Hathway, 1978a).

Anderson et al. (1979a) studied the rate of uptake of vinylidene chloride vapor by fasted male rats. Fasting (for 18 hours) has been shown to deplete the glutathione content of the liver, and to increase the sensitivity of rats to the toxicity of vinylidene chloride; these observations are consistent with the role of glutathione in the detoxification of vinylidene chloride (Section 10.1.2.2.1). Uptake was determined from disappearance of vinylidene chloride from a closed chamber as a function of time, corrected for nonspecific loss.

Uptake could be resolved into a rapid and a slow phase. The rapid phase was not affected by pretreatment of the rats with inhibitors of microsomal metabolism and could therefore be separated from the slow phase, in which the rate was markedly reduced by these inhibitors (the slow phase is discussed in Section 10.1.2.1). The rapid phase was a first-order process with a rate constant of 2.2 $hr^{-1}$ (half-life = 0.315 hr). The rate constant did not vary significantly with initial concentration of vinylidene chloride. The magnitude of the rapid component was proportional to the initial concentration of vinylidene chloride and the weight of rats in the chamber. The concentration of vinylidene chloride in the body as a function of concentration in the chamber at equilibrium (whole body solubility coefficient) was 0.0163 mg/kg body weight/ppm chamber, equivalent to a body/gas distribution coefficient of 4.04. The rapid phase was essentially complete in 60 to 80 minutes. On the basis of these characteristics, Andersen et al. (1979a) concluded that the rapid phase represented whole body equilibration.

Jaeger et al. (1977a) investigated the distribution of radioactivity in various tissues 30 minutes after cessation of a 2-hour exposure of fed and fasted male rats to $^{14}C$-vinylidene chloride. Exposure occurred in a closed chamber; the initial concentration of vinylidene chloride was 2000 ppm. Although there was no significant difference between fed and fasted rats in the overall rate of uptake of vinylidene chloride, the tissues and serum of fasted rats contained more radioactivity than those of fed rats. The highest concentration of radioactivity was found in kidney, and the next highest in liver. Spleen contained an intermediate level of radioactivity; heart and brain contained the least. In both fed and fasted rats, most of the radioactivity in kidney was trichloroacetic acid (TCA)-soluble. Approximately one-third of the radioactivity in liver was TCA-precipitable, indicating that it was covalently bound to macromolecules. Liver contained more TCA-precipitable radioactivity than did kidney.

McKenna et al. (1978a) from the Dow Chemical Company investigated the fate of $^{14}C$-vinylidene chloride in fed and fasted (deprived of food for 18 hours) male rats 72 hours after a 6-hour inhalation exposure to 10 or 200 ppm. As presented in Table 10-1, fasted rats had lower body burdens of $^{14}C$ than did fed rats. Although most of the body burdens had been excreted within 72 hours, a small but significant amount remained in the carcasses. After the 200 ppm exposure, more radioactivity was retained in the carcasses of fasted rats than in those of fed rats. All of the radioactivity found in urine, feces, and tissues was nonvolatile and therefore was considered to represent nonvolatile metabolites of vinylidene chloride. Seventy-two hours after exposure to either 10 or 200 ppm vinylidene chloride, concentrations of radioactivity were highest in kidney and liver and decreased in the following order: kidney > liver > lung > skin > plasma > fat, muscle, and remaining carcass. At both exposure levels, concentrations of radioactivity remaining in the tissues of fasted rats were greater than those in the tissues of fed rats when the data were normalized to account for the lower end-exposure body burdens of fasted rats.

In similar experiments with intragastric administration of 1 or 50 mg $^{14}C$-vinylidene chloride to fed and fasted male rats, McKenna et al. (1978b) found at 72 hours after dosing that concentrations of radioactivity were highest in liver and next highest in kidney. Levels of radioactivity in lung were not as high as noted in the inhalation experiment previously described (McKenna et al., 1978a). Jones and Hathway (1978a) reported that whole animal autoradiography of young male rats after intragastric administration of $^{14}C$-vinylidene chloride revealed high concentrations of $^{14}C$ in the kidneys and liver 30 minutes after dosing, followed at 1 hour by a more general distribution of radioactivity throughout the soft tissues. According to the authors, liver and kidney retained radioactivity longer than did other tissues.

## TABLE 10-1

End-Exposure Body Burdens and Disposition of $^{14}$C Activity in Rats 72 Hours Following Inhalation Exposure to 10 or 200 ppm of $^{14}$C-Vinylidene Chloride for 6 Hours[a]

| | 10 ppm | | 200 ppm | |
|---|---|---|---|---|
| | Fasted rats | Fed rats | Fasted rats | Fed rats |
| Body burden[b] | | | | |
| Total milligram equivalents of $^{14}$C-vinylidene chloride/rat | 0.589 ± 0.19[c] | 0.748 ± 0.041 | 9.57 ± 0.08[c] | 11.41 ± 0.64 |
| Milligram equivalents of $^{14}$C-vinylidene chloride/kg | 2.30 ± 0.05 | 2.89 ± 0.12 | 35.39 ± 0.30[c] | 44.53 ± 0.64 |
| Percentage body burden | | | | |
| Expired vinylidene chloride | 1.60 ± 0.19 | 1.63 ± 0.07 | 8.36 ± 0.27[c] | 4.17 ± 0.62[d] |
| $^{14}CO_2$ | 8.27 ± 0.80 | 8.74 ± 1.86 | 7.24 ± 0.51 | 8.22 ± 0.54 |
| Urine | 78.19 ± 1.42 | 74.72 ± 1.65 | 70.41 ± 1.86 | 74.66 ± 2.62 |
| Feces | 6.75 ± 0.56[c] | 9.73 ± 0.05 | 2.72 ± 0.41 | 6.39 ± 1.50 |
| Tissues and carcass | 3.91 ± 0.05 | 3.45 ± 0.30 | 7.45 ± 1.01[c] | 4.26 ± 0.09[d] |
| Skin | 1.37 ± 0.25 | 1.30 ± 0.09 | 3.07 ± 1.0 | 1.92 ± 0.29 |
| Cage wash | 0.27 ± 0.11 | 0.44 ± 0.14 | 0.76 ± 0.10[c] | 0.34 ± 0.02 |

[a] Source: McKenna et al., 1978a

[b] The values are given as X ± SE (n = four per group).

[c] Significantly different from fed animals of the same exposure group, $p<0.05$, Student's t test.

[d] Significantly different from fed animals of the 10 ppm exposure group, $p<0.05$, Student's t test.

### 10.1.2 Metabolism

The metabolism of vinylidene chloride by experimental animals has been extensively studied. A metabolic scheme that integrates the data from several laboratories is presented in Figure 10-1. In this scheme, vinylidene chloride is metabolized to the following reactive intermediates: 1,1-dichloroethylene oxide, chloroacetyl chloride, and monochloroacetic acid. These reactive intermediates are detoxified by conjugation with glutathione (GSH), ultimately leading to the formation of mercapturic acids (derivatives of N-acetyl cysteine) or degradation to various thioglycolic acids. A minor pathway for detoxification of monochloroacetic acid involves further oxidation to $CO_2$. 1,1-Dichloroethylene oxide, chloroacetyl chloride, and monochloroacetic acid can react with (i.e., alkylate) cellular macromolecules; this type of reaction could be involved in the production of toxic effects.

From an analysis of the literature on the metabolism of chlorinated ethylenes, Loew et al. (1983) have concluded that the extent of metabolism of these compounds generally decreases with increasing degree of chlorine substitution. Thus the extent of metabolism appears to be: vinyl chloride ≈ vinylidene chloride > trans or cis-dichloroethylene > trichloroethylene > tetrachloroethylene. These conclusions were drawn from a comparison of the results of a number of oral and inhalation studies, taking into account species and dose differences.

#### 10.1.2.1 Metabolism to Reactive Intermediates

Vinylidene chloride is thought to be metabolized by the microsomal mixed-function oxidase system to an epoxide (oxirane), 1,1-dichloroethylene oxide (Bonse and Henschler, 1976; Liebman and Ortiz, 1977; McKenna et al., 1977). According to Bonse and Henschler (1976), 1,1-dichloroethylene oxide is extremely unstable and could not be isolated during attempts at chemical synthesis,

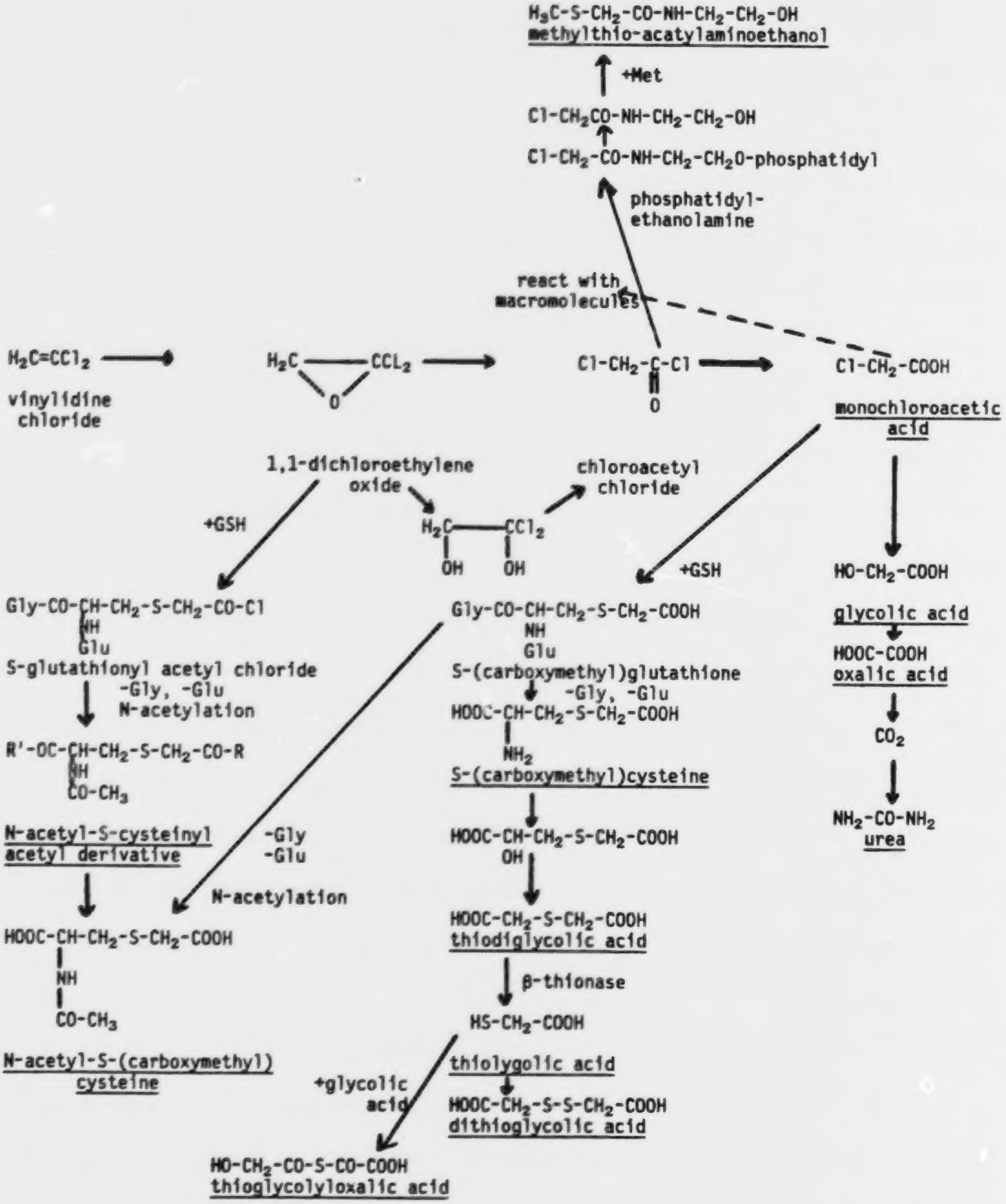

H3C-S-CH2-CO-NH-CH2-CH2-OH
methylthio-acatylaminoethanol
+Met
Cl-CH2CO-NH-CH2-CH2-OH
Cl-CH2-CO-NH-CH2-CH2O-phosphatidyl
phosphatidyl-ethanolamine
react with macromolecules
H2C=CCl2
vinylidine chloride
H2C—CCL2 O
Cl-CH2-C-Cl O
Cl-CH2-COOH
monochloroacetic acid
1,1-dichloroethylene oxide
chloroacetyl chloride
+GSH
H2C—CCl2 OH OH
+GSH
HO-CH2-COOH
glycolic acid
HOOC-COOH
oxalic acid
CO2
NH2-CO-NH2
urea
Gly-CO-CH-CH2-S-CH2-CO-Cl NH Glu
S-glutathionyl acetyl chloride
-Gly, -Glu
N-acetylation
R'-OC-CH-CH2-S-CH2-CO-R NH CO-CH3
N-acetyl-S-cysteinyl acetyl derivative
Gly-CO-CH-CH2-S-CH2-COOH NH Glu
S-(carboxymethyl)glutathione
-Gly, -Glu
HOOC-CH-CH2-S-CH2-COOH NH2
S-(carboxymethyl)cysteine
-Gly
-Glu
N-acetylation
HOOC-CH-CH2-S-CH2-COOH OH
HOOC-CH2-S-CH2-COOH
thiodiglycolic acid
β-thionase
HS-CH2-COOH
thiolygolic acid
HOOC-CH2-S-S-CH2-COOH
dithioglycolic acid
HOOC-CH-CH2-S-CH2-COOH NH CO-CH3
N-acetyl-S-(carboxymethyl) cysteine
+glycolic acid
HO-CH2-CO-S-CO-COOH
thioglycolyloxalic acid

Figure 10-1. Metabolic Pathways for Vinylidene Chloride. Identified metabolites are underlined. Identification was usually by gas chromatography-mass spectrometry. R' is considered to be OH; R is unknown. (Adapted from Jones and Hathway, 1978a, 1978b; Reichert et al., 1979; Leibman and Ortiz, 1977).

although its expected rearrangement product, chloroacetyl chloride, was formed after chemical oxidation of vinylidene chloride with m-chloroperbenzoic acid. Metabolism of vinylidene chloride to this epoxide has not been directly demonstrated, but rather is inferred by analogy with the metabolism of other chlorinated ethylenes (Leibman and Ortiz, 1977; Bonse and Henschler, 1976; Uehleke et al., 1976) and from the observed metabolism of vinylidene chloride to monochloroacetic acid.

The metabolism of vinylidene chloride by hepatic cytochrome P-450 in microsomes from male Long-Evans rats was studied _in vitro_ by Costa and Ivanetich (1982) in an attempt to identify metabolites and detoxification pathways. Vinylidene chloride was shown to be metabolized to dichloroacetaldehyde and to monochloroacetic acid by hepatic microsomes, after binding to cytochrome P-450 in the presence of an NADPH-generating system. Certain forms of cytochrome P-450 (such as the phenobarbital-inducible form) appear to play minor roles in the metabolism of vinylidene chloride, while other forms may be more efficient.

Radioactively-labeled monochloroacetic acid has been identified in the urine of rats after intragastric administration in corn oil of $^{14}$C-vinylidene chloride (Jones and Hathway, 1978a), in rat livers perfused with $^{14}$C-vinylidene chloride (Reichert and Bashti, 1976), and after incubation of $^{14}$C-vinylidene chloride with 9000 x g supernatants from rat liver homogenates (Leibman and Ortiz, 1977). Monochloroacetic acid is the expected product of rearrangement of the epoxide to chloroacetyl chloride and subsequent hydrolysis (Bonse and Henschler, 1976; Leibman and Ortiz, 1977). Alternatively, monochloroacetic acid could result from metabolism of the epoxide by epoxide hydrase to the diol shown in Figure 10-1, followed by rearrangement to chloroacetyl chloride and hydrolysis to monochloroacetic acid (Leibman and Ortiz, 1977).

The relative importance of the diol pathway is difficult to assess. Jones and Hathway (1978b) suggested that conversion to the diol is not important because $CO_2$, a possible degradation product, is found only in small amounts *in vivo*. It is not known, however, whether the diol is metabolized to $CO_2$ to a significant extent. Leibman and Ortiz (1977) concluded that diol formation is relatively unimportant in the metabolism of vinylidene chloride to monochloroacetic acid because 1,1,1-trichloropropene-2,3-oxide and cyclohexene oxide (inhibitors of epoxide hydrase) stimulated, rather than inhibited, the formation of monochloroacetic acid in incubations of vinylidene chloride with 9000 x g supernatants from rat liver homogenates. These two inhibitors react with GSH (Oesch and Daly, 1972). Thus the results of Leibman and Ortiz (1977) could be explained by the depletion of GSH, thereby resulting in the accumulation of monochloroacetic acid. Furthermore, according to Oesch (1972), there are two types of epoxide hydrase activity--one is tightly coupled with mixed-function oxidase activity, whereas the other is not. The coupled form is thought to be more important in detoxifying epoxides formed by mixed function oxidases and is relatively resistant to inhibitors (Oesch, 1972).

Andersen et al. (1979a) investigated the metabolism of vinylidene chloride by studying the rate of uptake of vinylidene chloride vapor by fasted male rats. Uptake was determined from the disappearance of vinylidene chloride as a function of time (usually plotted on linear coordinates) from a closed chamber, corrected for nonspecific loss. The uptake of vinylidene chloride by fasted male rats was biphasic; the initial rapid phase represented tissue equilibration (Section 10.1.1) and the slow phase represented metabolism. The slow phase of uptake was relatively independent of concentration at higher exposure levels and dependent on concentration at lower exposures. A series of uptake experiments using different initial concentrations of vinylidene chloride were performed. The instantaneous rates of uptake were determined from tangents to the curves at 60 to 80 minutes of exposure, so that the contribution from the rapid phase was negligible. Instantaneous rates plotted against actual chamber concentrations yielded a rectangular hyperbola typical of Michaelis-Menten kinetics, as would be expected if the slow phase represented metabolism. $V_{max}$, the maximum velocity at saturating exposure levels, was 132 ppm/kg/hr (equivalent to 15.87 mg of vinylidene chloride metabolized/kg/hr). $K_m$, the exposure level that produced half the maximum velocity, was 335 ppm (equivalent to a body burden of 5.43 mg/kg).

At an initial concentration of vinylidene chloride of 300 ppm in air (below the $K_m$), the rate constant of the slow phase was reduced 84, 92, and 65% by pretreatment with pyrazole, carbon tetrachloride, and aminotriazole, respectively, but was unaffected by pretreatment with SKF 525A or 2,3-epoxypropan-1-ol. These compounds, with the exception of 2,3-epoxypropan-1-ol, are inhibitors of microsomal metabolism; all have been shown to affect the acute toxicity of vinylidene chloride (Section 10.2) (Andersen et al., 1980). At high initial concentrations of vinylidene chloride, the $V_{max}$ was unaffected by pretreatment with phenobarbital or by using fed rather than fasted rats, and was similar for immature and mature rats (Andersen et al., 1979a).

As determined by Andersen et al. (1979b), the acute toxicity of inhaled vinylidene chloride was relatively independent of concentration but dependent on duration of exposure at concentrations above 200 ppm (Section 10.2). In an attempt to correlate metabolism with toxicity, Andersen et al. (1979a) calculated the two theoretical curves shown in Figure 10-2. The solid line is based on the assumption that the product of exposure concentration times the duration of exposure required to produce a given effect is constant. The dashed line is based on the assumption that the product of the rate of metabolism (at each

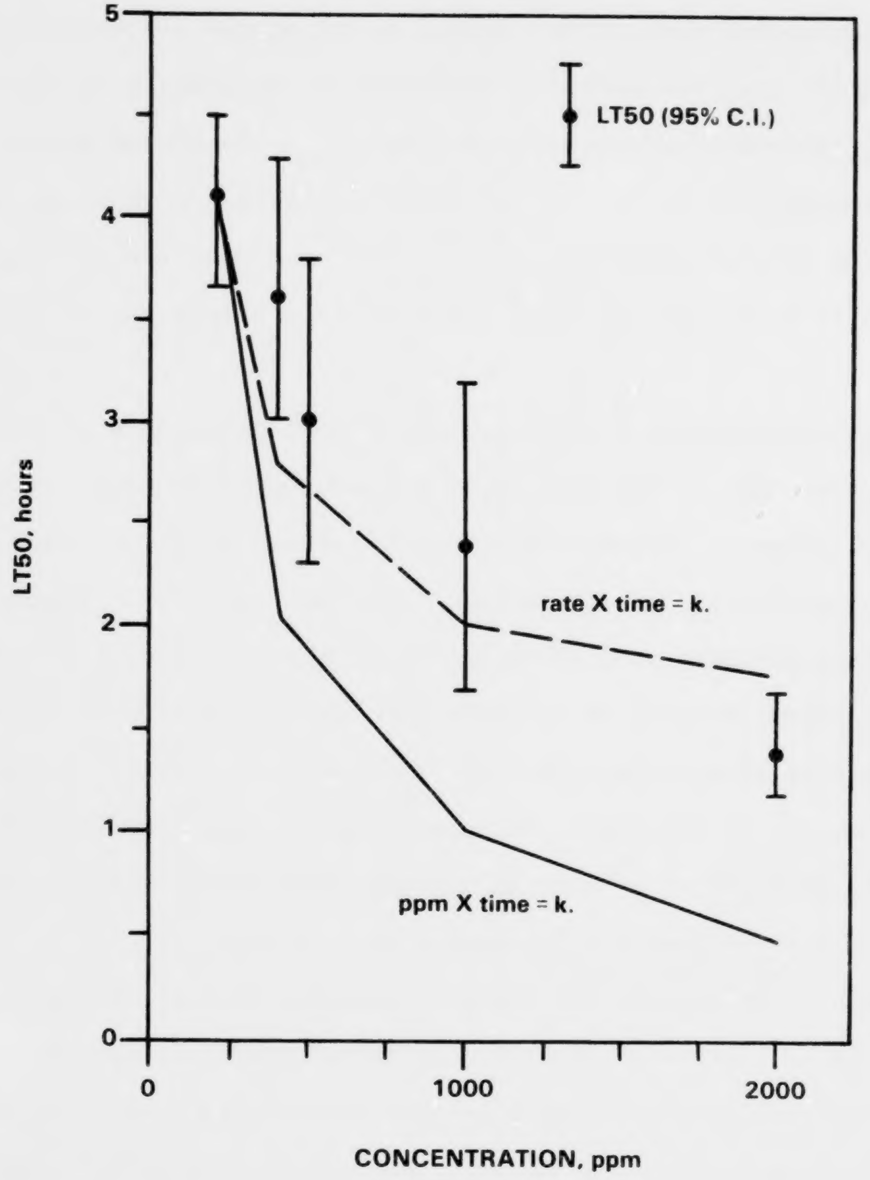


Figure 10-2. Comparisons of observed LT50 data for vinylidene chloride with theoretical curves predicted for two different mechanisms of toxicity. As indicated by the symbol in upper right, data are given as LT50 and its 95% confidence interval.

Source: Andersen (1979a).

exposure concentration) times the duration of exposure required to produce a given effect is constant. The experimentally determined $LT_{50}$ (time required to produce 50% mortality rate) of 4.1 hours at a 200 ppm exposure concentration was used to calculate the constant. The experimentally determined $LT_{50}$ values (Andersen et al., 1979b) fit the theoretical curve based on metabolism more closely than they fit the theoretical curve based on exposure concentration, suggesting that toxicity is a function of the amount of metabolite formed rather than the concentration of vinylidene chloride (Andersen et al., 1979a).

Using an experimental protocol similar to that of Andersen et al. (1979a), Filser and Bolt (1979) investigated the kinetics of metabolism of vinylidene chloride in the male rat. Filser and Bolt (1979) focused on strict zero-order and first-order kinetics, whereas Andersen et al. (1979a) focused on Michaelis-Menten kinetics. Both groups of investigators, however, demonstrated the saturable dose-dependent nature of vinylidene chloride metabolism. As determined by Filser and Bolt (1979), the $V_{max}$ (velocity for zero-order metabolism) was 100 μmol/kg/hr (9.7 mg/kg/hr).

Reichert and Henschler (1978) reported that pretreatment of female rats with an inducer of mixed-function oxidases, DDT, increased the uptake of vinylidene chloride 35%, while direct addition of inhibitors (pyrazole and ethanol) of mixed-function oxidases decreased the uptake of vinylidene chloride 35 to 40% in isolated, perfused rat livers. Pretreatment with phenobarbital, or direct addition of SKF 525A, 6-nitro-1,2,3-benzothiadiazole, and 5,6-dimethyl-1,2,3-benzothiadiazole to the perfusate had little effect on uptake when low concentrations of vinylidene chloride were used. The uptake of vinylidene chloride, measured as the difference between prehepatic and posthepatic concentrations of vinylidene chloride under steady state conditions, was taken to be equivalent to metabolism. The authors stated that with a high concentration of vinylidene chloride in the perfusate, pretreatment with phenobarbital elevated the uptake of vinylidene chloride by approximately 30%. The results of Reichert and Henschler (1978) for perfused livers of female rats are in general agreement with the results of Andersen et al. (1979a) for intact fasted male rats except for the stimulation of uptake (metabolism) by phenobarbital pretreatment noted by Reichert and Henschler (1978). Pretreatment with ethanol for a 3-week period enhanced the activity of rat liver drug-metabolizing enzymes six-fold in their ability to convert vinylidene chloride (Sato et al., 1980). The authors discussed the increased rate of vinylidene chloride metabolism and noted that this may increase the toxicity of the compound if metabolites are the actual causative agents.

A comparison of sex, age, and species differences in metabolism and toxicity suggests that vinylidene chloride is metabolized to a toxic intermediate by mixed-function oxidases. In general, microsomal oxidation of compounds was found to be greater in male rats than in female rats (Kato, 1974). Male rats were much more susceptible to the hepatotoxic effects of vinylidene chloride than were female rats (Andersen and Jenkins, 1977). Microsomal oxidase activity reached a maximum in the rat at 30 to 40 days of age (Kato et al., 1964), an age range which corresponded to the size (100-150 g) of rats most susceptible to the toxicity of vinylidene chloride in the report of Andersen and Jenkins (1977). All of the studies reviewed in Section 10.1 of this report, however, were performed on mature (180-350 g) rats and mature mice unless otherwise specified.

A greater percentage of an orally administered dose (50 mg/kg) of $^{14}C$-vinylidene chloride was metabolized by mice than by rats, based on quantitation of excreted radiolabeled metabolites and unchanged vinylidene chloride (Jones and Hathway, 1978b). Similarly, as presented in Table 10-2, mice metabolized more vinylidene chloride per kg body weight than did rats after inhaling 10 ppm

## TABLE 10-2

## End-Exposure Body Burdens and Disposition of $^{14}C$-Activity in Rats and Mice 72 Hours Following Inhalation Exposure to 10 ppm $^{14}C$-Vinylidene Chloride for 6 Hours[a]

| | Mice[d] | Rats[e] |
|---|---|---|
| Body burden, mg-Eq $^{14}C$-vinylidene chloride/kg | 5.30 ± 0.75 | 2.89 ± 0.24 |
| Total metabolized vinylidene chloride mg-Eq $^{14}C$-vinylidene chloride/kg[c] | 5.27 ± 0.74 | 2.84 ± 0.26 |
| Percentage body burden[b] | | |
| Expired vinylidene chloride | 0.65 ± 0.07 | 1.63 ± 0.14 |
| Expired $^{14}CO_2$ | 4.64 ± 0.17 | 8.74 ± 3.72 |
| Urine | 80.83 ± 1.68 | 74.72 ± 2.30 |
| Feces | 6.58 ± 0.81 | 9.73 ± 0.10 |
| Carcass | 5.46 ± 0.41 | 4.75 ± 0.78 |
| Cage Wash | 1.83 ± 0.84 | 0.44 ± 0.28 |

[a] Source: McKenna et al., 1977

[b] X ± SE, n = 4.

[c] Calculated from the total $^{14}C$-activity recovered (end-exposure body burden) minus the $^{14}C$-vinylidene chloride exhaled from each rat

[d] Mice were males of the Ha(ICR) strain.

[e] Rats were males of the Sprague-Dawley strain.

$^{14}$C-vinylidene chloride in air for 6 hours (McKenna et al., 1977). The overall pattern of excretion for mice and rats was similar. The concentration of covalently-bound radioactivity in liver and kidney was much higher in mice than in rats, as shown in Table 10-3. Binding was measured as radioactivity that was TCA-precipitable and 80% methanol-insoluble and thus represented radioactivity covalently bound to protein and nucleic acid. As discussed in Section 10.4.1, the 9000 x g supernatant from mouse liver was more active than the 9000 x g supernatant from rat liver in metabolizing vinylidene chloride to mutagenic substances. These observations of increased metabolism and covalent binding in mice versus rats parallel the greater sensitivity of mice to the acute lethality, hepatotoxicity, and renal toxicity of vinylidene chloride (Short et al., 1977a,b). A brief review of the metabolism of vinylidene chloride by rats and mice is presented by Cooper (1980).

Hypoxia (7% oxygen), which inhibits mixed-function oxidase activity, decreased the hepatotoxicity of inhaled vinylidene chloride to fasted male rats (Jaeger, 1978). Disulfuram, which has been reported to inhibit hepatic mixed-function oxidases (Zemaitis and Green, 1976), protected male mice from the acute lethal and hepatotoxic effects of inhaled vinylidene chloride (Short et al., 1977a,b). Measured at 4 and 24 hours after intraperitoneal (i.p.) administration of $^{14}$C-vinylidene chloride, covalent binding of radioactivity to protein in liver and kidney was significantly reduced in mice fed disulfuram before and during exposure (Short et al., 1977a,b). Additional studies dealing with the relationship between the induction and inhibition of microsomal mixed function oxidases and the toxicity of vinylidene chloride are discussed in Section 10.2.

The identification of methylthio-acetylaminoethanol as a urinary metabolite of vinylidene chloride suggested the alkylation of lipids by a reactive intermediate (Reichert et al., 1979; Henschler and Hoos, 1981). The pathway of

### TABLE 10-3

### Covalently Bound $^{14}$C-Activity in Tissues 72 Hours Following Inhalation Exposure to 10 ppm $^{14}$C-Vinylidene Chloride for 6 Hours[a]

| | $^{14}$C-vinylidene chloride, µg-Eq/g protein ($\bar{X} \pm$ SE, n = 4) | |
|---|---|---|
| | Liver | Kidney |
| Mice | 22.29 ± 3.77 | 79.55 ± 19.11 |
| Rats | 5.28 ± 0.14 | 13.14 ± 1.15 |

[a] Source: McKenna et al., 1977

formation of methylthio-acetylaminoethanol has not yet been clarified. Reichert et al. (1979) hypothesized that the first step could be the reaction of chloroacetyl chloride with phosphatidyl ethanolamine (a constituent of lipid membranes). This postulated pathway is illustrated in Figure 10-1.

#### 10.1.2.2 Detoxification Pathways

##### 10.1.2.2.1 Conjugation with Glutathione

Conjugation of vinylidene chloride or its metabolites with glutathione is indicated because administration of $^{14}$C-vinylidene chloride (intragastrically or by inhalation) to rats and mice produced several S-containing radioactive metabolites. The major urinary metabolites of vinylidene chloride in rats have been identified as thiodiglycolic acid (thiodiacetic acid) (Jones and Hathway, 1978a,b; Reichert et al., 1979; McKenna et al., 1977, 1978a,b) as well as the following mercapturic acids: an N-acetyl-S-cysteinyl acetyl derivative shown in Figure 10-1 (Jones and Hathway, 1978a,b), N-acetyl-S-(carboxymethyl)cysteine (Reichert et al., 1979), and N-acetyl-S-(2-hydroxyethyl)cysteine (McKenna et al., 1977, 1978a,b). N-acetyl-S-(2-hydroxyethyl)cysteine is not shown as part of the metabolic scheme in Figure 10-1 because its relationship to other metabolites of vinylidene chloride is unclear. The major urinary metabolites of vinylidene chloride in mice were dithioglycolic acid and an N-acetyl-S-cysteinyl acetyl derivative shown in Figure 10-1 (Jones and Hathway, 1978b).

Vinylidene chloride itself does not appear to react with glutathione. McKenna et al. (1977) stated that they were unable to demonstrate conjugation of vinylidene chloride with GSH either nonenzymatically or in the presence of the soluble fraction (which contains glutathione S-transferases) of a rat liver homogenate. Conjugation with GSH required the presence of a microsomal enzyme system, suggesting that vinylidene chloride was metabolized to a reactive intermediate before conjugation with glutathione. Unfortunately, no data or experimental details were presented.

Additional evidence that the S-containing metabolites (mercapturic and thioglycolic acids) of vinylidene chloride arise from conjugation with GSH is as follows. The origin of the cysteine moiety in mercapturic acid derivatives of many xenobiotics has been shown to be GSH (Chasseaud, 1973). In vivo experiments with unlabeled vinylidene chloride, in which the cysteine-cystine pools were labeled with $^{14}C$, resulted in the production of labeled thiodiglycolic acid (Jones and Hathway, 1978a). Thus, at least part of the carbon skeleton of thiodiglycolic acid must have been derived from cysteine.

Administration of vinylidene chloride to male or female rats in vivo, or during perfusion of isolated rat livers, depleted the liver glutathione content (Jaeger et al., 1974; Reichert et al., 1978; McKenna et al., 1977; McKenna, 1979). Depletion of GSH was maximal at about 4 hours after oral administration of vinylidene chloride to female rats and was dependent on the dose administered (Reichert et al., 1978). Depletion of glutathione after inhalation exposure of male rats was similarly dose-dependent (McKenna et al., 1977; McKenna, 1979).

Reichert et al. (1978) attempted to demonstrate a correlation between the GSH content of isolated, perfused livers from female rats and the rate of metabolism of vinylidene chloride measured as uptake (see Section 10.1.2.1, Reichert and Henschler, 1978). When the GSH content was decreased 80% by the prior addition of diethyl maleate to the perfusate, an 18% decrease in vinylidene chloride metabolism was measured. A less pronounced decrease in GSH content, produced by prior fasting of the rats, was not associated with a decrease in vinylidene chloride metabolism; a slight increase (10%) was observed, although neither change in metabolism was statistically significant. The authors concluded that the GSH content of liver becomes a limiting factor in the metabolism of vinylidene chloride only when the GSH content has been severely depleted.

Evidence for the metabolic pathways shown in Figure 10-1 that involve conjugation with GSH has been obtained from the analysis of the urinary metabolites resulting from the administration of $^{14}C$-labeled vinylidene chloride, monochloroacetic acid, and other metabolic intermediates to rats and mice. As shown in Table 10-4, the N-acetyl-S-cysteinyl acetyl derivative was detected only when rats were given $^{14}C$-vinylidene chloride and not when they were given $^{14}C$-monochloroacetic acid. The N-acetyl-S-cysteinyl acetyl derivative must therefore have come from conjugation of GSH with a metabolite arising prior to the formation of monochloroacetic acid (Jones and Hathway, 1978a).

The administration of either $^{14}C$-vinylidene chloride or $^{14}C$-monochloroacetic acid gave rise to $^{14}C$-labeled S-(carboxymethyl)cysteine, N-acetyl-S-(carboxy methyl)cysteine, thiodiglycolic acid, thioglycolic acid, and dithioglycolic acid (Tables 10-4 and 10-5, Hathway, 1977; Jones and Hathway, 1978a). These results suggest that monochloroacetic acid is a key intermediate in the metabolism of vinylidene chloride and the S-(carboxymethyl)glutathione can be metabolized to thioglycolic acids or to a mercapturic acid.

S-(Carboxymethyl)cysteine was metabolized to thiodiglycolic acid and to $CO_2$ (Green and Hathway, 1977; Yllner, 1971; Jones and Hathway, 1978b). Because labeled $CO_2$ was detected only when the cysteine moiety of S-(carboxymethyl)-cysteine was uniformly labeled with $^{14}C$ (Jones and Hathway, 1978b) but not when the carboxymethyl moiety was labeled (Yllner, 1971), $CO_2$ must have been produced from the decarboxylation of cysteine. $^{14}C$-Thiodiglycolic acid was converted to thioglycolic acid, dithioglycolic acid, and thioglycolyloxalic acid, indicating that these metabolites arise from β-thionase hydrolysis of thiodiglycolic acid (Jones and Hathway, 1978b), shown in Figure 10-1. As can be seen from the relative proportions of thioglycolic acids in Table 10-5, β-thionase conversion occurred to a greater extent in mice than in rats (Jones and Hathway, 1978b).

## TABLE 10-4

### Relative Proportions of $^{14}$C Urinary Metabolites After Intragastric Administration of 350 mg/kg 1-$^{14}$C-Vinylidene Chloride or 50 mg/kg $^{14}$C-Monochloroacetic Acid to Male Rats[a]

| Vinylidene Chloride and Chloroacetic Acid Metabolites | % of Urinary Radioactivity Vinylidene Chloride | Chloroacetic Acid |
|---|---|---|
| Thiodiglycolic acid | 37.0 | 90.0 |
| N-acetyl-S-cysteinyl-acetyl derivative | 48.0 | 0 |
| Dithioglycolic acid | 5.0 | 3.0 |
| Thioglycolic acid | 3.0 | 3.0 |
| Chloroacetic acid | 3.0 | 0 |
| Urea | 0.5 | 0.5 |
| S-(Carboxymethyl)cysteine | 0 | 2.0 |
| N-acetyl-S-(carboxymethyl)cysteine | -- | 2.0* |

[a] Source: Hathway, 1977*; Jones and Hathway, 1978a

## TABLE 10-5

### Relative Proportions of $^{14}$C-Excretory Products After Intragastric Administration of 50 mg/kg of 1-$^{14}$-C-Vinylidene Chloride to Male Rats or Mice[a]

| $^{14}$C Excretory Products | $^{14}$C Expressed as Percent of Dose | |
|---|---|---|
| | Mice | Rats |
| Unchanged vinylidene chloride (pulmonary excretion) | 6 | 28 |
| Carbon dioxide (pulmonary excretion) | 3 | 3.5 |
| Chloroacetic acid | 0 | 1 |
| Thiodiglycolic acid | 3 | 22 |
| Thioglycolic acid | 5 | 3 |
| Dithioglycolic acid | 23 | 5 |
| Thioglycolyloxalic acid | 3 | 2 |
| N-Acetyl-S-cysteinyl acetyl (derivative) | 50 | 28 |
| N-Acetyl-S-(2-carboxymethyl) cysteine | 4 | 0 |
| Urea | 3 | 3.5 |

[a] Source: Jones and Hathway, 1978b

Results of experiments with rats suggest that conjugation with glutathione serves to detoxify reactive metabolites of vinylidene chloride. The hepatotoxicity of vinylidene chloride in vivo and in the isolated perfused liver was greater if vinylidene chloride was administered when GSH levels in the liver had been diminished by fasting (Jaeger et al., 1974, 1975a; McKenna et al., 1978a), diurnal variation (Jaeger et al., 1973a, 1975a), by pretreatment with diethyl maleate (Jaeger et al., 1974, 1975a; Andersen et al., 1980), or by pretreatment with thyroxine (Jaeger et al., 1977b). The degree to which pretreatment with various epoxides or diethyl maleate exacerbated the acute toxicity of vinylidene chloride was related to the degree to which these compounds depleted GSH (Andersen et al., 1980). Increasing the levels of GSH in the liver by chemical or surgical thyroidectomy protected against the toxicity of vinylidene chloride (Jaeger et al., 1977b). Hypoxia, which inhibited mixed-function oxidase activity, protected against the hepatotoxicity of vinylidene chloride and concomitantly prevented depletion of liver GSH (Jaeger, 1978).

Additional evidence for the role of GSH in detoxification can be obtained from studies of covalent binding. Fasting and, hence, a low level of liver GSH were associated with decreased total metabolism of $^{14}$C-vinylidene chloride, increased covalent binding of radioactivity to liver macromolecules, and centrilobular hepatic necrosis in male rats (McKenna et al., 1978a,b). Data for intragastric and inhalation administration are shown in Tables 10-6 and 10-7, respectively. Binding was measured 72 hours after exposure, as radioactivity that was TCA-precipitable and 80% methanol-insoluble, and thus represents radioactivity covalently bound to protein and nucleic acid. At the higher exposure levels, fasted male rats had significantly greater concentrations of bound radioactivity in their livers when the data were normalized to account for differences in metabolism.

## TABLE 10-6

### Metabolism of $^{14}$C-Vinylidene Chloride and Covalent Binding of $^{14}$C Activity to Rat Hepatic Tissue after Intragastric Dose of $^{14}$C-Vinylidene Chloride[a,b]

| Dose | n | A<br>Metabolized vinylidene chloride (mg Eq of $^{14}$C-vinylidene chloride/kg) | B<br>Covalently bound vinylidene chloride (μg Eq of $^{14}$C-vinylidene chloride/g of liver protein) | B/A[c] |
|---|---|---|---|---|
| 1 mg/kg | | | | |
| Fed rats | 4 | 0.956 ± 0.136[d] | 0.854 ± 0.003 | 0.89 ± 0.36 |
| Fasted rats | 4 | 0.924 ± 0.032 | 0.861 ± 0.022 | 0.93 ± 0.04 |
| 50 mg/kg | | | | |
| Fed rats | 3 | 39.08 ± 3.58 | 37.67 ± 3.16 | 0.96 ± 0.12 |
| Fasted rats | 3 | 31.86 ± 1.81[e] | 44.45 ± 3.21 | 1.40 ± 0.08[e] |

[a] Source: McKenna et al., 1978b

[b] All values represent the $\bar{X} \pm SE$ for the number of rats indicated in the table.

[c] Covalent binding data normalized to account for differences in metabolized vinylidene chloride.

[d] Calculated from the total recovery of $^{14}CO_2$ plus nonvolatile radioactivity from each animal.

[e] Significant fed vs. fasted difference, $p<0.05$, Student's t test.

TABLE 10-7

Metabolism of $^{14}$C-Vinylidene Chloride and Covalent Binding of $^{14}$C Activity to Rat Hepatic Tissue after Inhalation Exposure to 10 or 200 ppm of $^{14}$C-Vinylidene Chloride[a]

| Exposure concentration and pretreatment | Body burden (mg of $^{14}$C-vinylidene chloride/kg) | A<br>Metabolized $^{14}$C-vinylidene chloride (mg/Eq/kg) | B<br>Microgram equivalents of $^{14}$C-vinylidene chloride bound per gram of liver protein | B/A[b] |
|---|---|---|---|---|
| 10 ppm | | | | |
| Fed | 2.89 ± 0.12[c] | 2.84 ± 0.13[d] | 2.49 ± 0.17 | 0.88 ± 0.08 |
| Fasted | 2.30 ± 0.05 | 2.26 ± 0.06 | 2.47 ± 0.29 | 1.10 ± 0.15 |
| 200 ppm | | | | |
| Fed | 44.53 ± 6.05 | 42.73 ± 3.18 | 64.18 ± 7.97 | 1.49 ± 0.10 |
| Fasted | 35.93 ± 0.30[e] | 32.92 ± 0.32[e] | 79.46 ± 4.90 | 2.42 ± 0.17[e] |

[a] Source: McKenna et al., 1978a

[b] Covalent binding data normalized to account for differences in metabolized vinylidene chloride.

[c] All values represented the mean ± SE for four rats.

[d] Calculated from the total $^{14}$C activity recovered (end-exposure body burden) minus the $^{14}$C-vinylidene chloride exhaled from each rat.

[e] Significantly different from fed rats exposed to the same concentration of vinylidene chloride, $p<0.05$, Student's t test.

Jaeger et al. (1977a) studied the subcellular distribution of free and bound radioactivity in livers from fed and fasted male rats exposed to $^{14}$C-vinylidene chloride. The analyses were performed 30 minutes after the cessation of a 2-hour inhalation exposure to vinylidene chloride (initial concentration, 2000 ppm) in a closed chamber. Significantly more radioactivity was found in all subcellular fractions from fasted animals than was found in those from fed animals. In livers from both fed and fasted rats, the radioactivity in the mitochondrial and microsomal fractions was largely TCA-precipitable; radioactivity in the cytoplasmic fractions was largely TCA-soluble. Because substantial amounts of the TCA-precipitable radioactivity of mitochondria and microsomes were soluble in chloroform, the authors suggested that binding to lipid had occurred. As discussed in Section 10.1.2.1, methylthio-acetylaminoethanol, a metabolite of vinylidene chloride, is though to be a product of the alkylation of lipids by a reactive intermediate of vinylidene chloride (Reichert et al., 1979; Henschler and Hoos, 1981). The amounts (per mg protein) of radioactivity presumably covalently bound to protein and nucleic acid (TCA-precipitable, chloroform-insoluble material) and to lipids (TCA-precipitable, chloroform-soluble material) were greater in mitochondrial and microsomal fractions from fasted rats than in the corresponding fractions from fed rats. Similarly, fasted rats had greater amounts (per mg protein) of radioactivity bound to protein and nucleic acid of the cytoplasmic fraction than did fed rats.

The relationship among GSH depletion, total metabolism, and covalent binding to macromolecules is not clear, as shown in Figure 10-3. Immediately after a 6-hour inhalation exposure to $^{14}$C-vinylidene chloride, male rats were sacrificed and hepatic GSH and covalent binding of radioactivity to protein and nucleic acids were measured. Apparently, the total amount of vinylidene chloride metabolized was measured, as indicated in the legend to Table 10-7. GSH

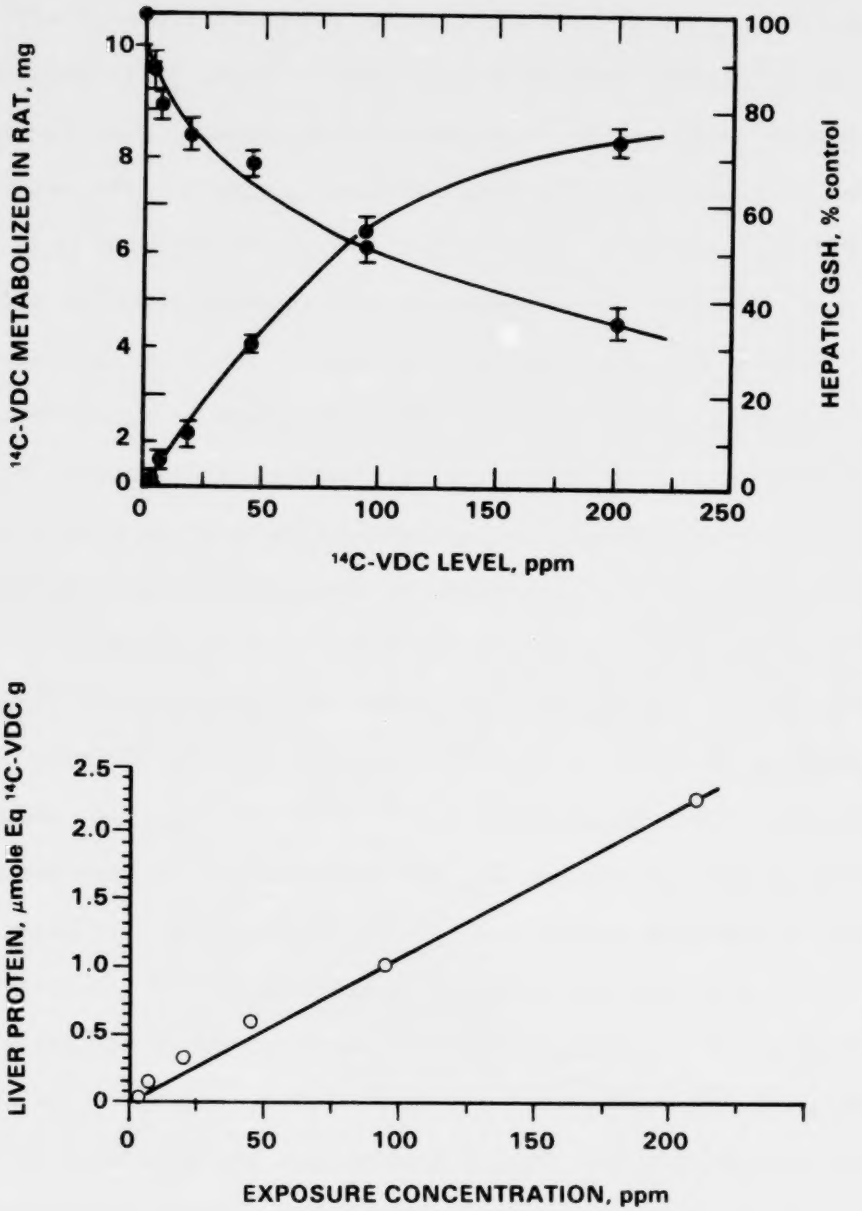


Figure 10-3. Dose-response relationship for hepatic glutathione (GSH) levels, total metabolism of $^{14}C$-vinylidene chloride (VDC) (top), and covalent binding of radioactivity to hepatic macromolecules (bottom).

Sources: Top: McKenna et al. (1977);
Bottom: McKenna et al. (1977), replotted by Dedrick (1979).

depletion and metabolism of vinylidene chloride displayed a similar, non-linear, dose dependence, whereas covalent binding increased linearly with increased exposure concentrations (McKenna et al., 1977; Dedrick, 1979; McKenna, 1979).

Suggestive evidence for an enhancement of hepatic lipid peroxidation *in vitro* after vinylidene chloride administration *in vivo* has been obtained in rats under conditions designed to overwhelm the GSH-conjugating capacity of the liver: i.e., the rats were pretreated with phenobarbital to induce mixed-function oxidases and were then treated with a very high dose of vinylidene chloride (500 mg/kg) (Siegers et al., 1982). This dose is higher than those used by other investigators to produce hepatic damage in noninduced rats (Section 10.2.1). In general, however, the hepatotoxic effects of vinylidene chloride are not thought to be due to a lipoperoxidative mechanism of action (Section 10.2.1).

#### 10.1.2.2.2 Minor Oxidative Pathway for Monochloroacetic Acid

Yllner (1971) demonstrated the oxidative degradation of $^{14}$C-monochloroacetic acid to glycolic acid, oxalic acid, and $CO_2$ by mice after i.p. administration. S-(Carboxymethyl-1,2-$^{14}$C)cysteine was not metabolized to appreciable amounts of labeled $CO_2$ and hence is not an intermediate in the metabolism of monochloroacetic acid to $CO_2$ (Yllner, 1971). Trace amounts of labeled oxalic acid have been detected in experiments with $^{14}$C-monochloroacetic acid in rats (Jones and Hathway, 1978a). Small amounts of labeled $CO_2$ and urea were identified as metabolites of $^{14}$C-vinylidene chloride (see Table 10-5) (Jones and Hathway, 1978b). Thus it appears that the metabolism of monochloroacetic acid to $CO_2$ is a minor pathway in the metabolism of vinylidene chloride.

### 10.1.3 Excretion

Vinylidene chloride and its metabolites are excreted fairly rapidly by experimental animals. The influence of route of administration and size of dose on the pattern of excretion of vinylidene chloride and its metabolites by male rats is shown in Table 10-8 (Jones and Hathway, 1978a). According to Jones and Hathway (1978a), 60% of the 0.5 mg/kg intravenous dose of $^{14}$C-vinylidene chloride was expired unchanged within 5 minutes of injection; 80% was expired unchanged within 1 hour. These results indicated an efficient arterial-alveolar transfer of vinylidene chloride. With intragastric or intraperitoneal administration of 0.5 mg/kg of $^{14}$C-vinylidene chloride, most of the radioactivity was excreted in the urine within the first 24 hours, whereas with 350 mg/kg, most of the radioactivity was expired as unchanged vinylidene chloride. The percentage of radioactivity expired as $CO_2$ decreased with the higher dose of vinylidene chloride. These changes in the pattern of excretion were attributed by Jones and Hathway (1978a) to saturable drug metabolism and to the efficient transfer of vinylidene chloride from systemic blood to the alveoli, leaving a relatively low concentration of vinylidene chloride available for metabolism in subsequent passes through the liver.

Similar dose-related shifts in the relative proportion of radioactivity excreted by various routes have been reported by Reichert et al. (1979) for intragastric administration of $^{14}$C-vinylidene chloride (0.5, 5.0, and 50 mg/kg) to female rats and by McKenna et al. (1978b) for intragastric administration of 1 and 50 mg/kg $^{14}$C-vinylidene chloride to fed and fasted male rats. Fed and fasted male rats that inhaled 200 ppm $^{14}$C-vinylidene chloride exhaled a greater percentage of their body burden as unchanged vinylidene chloride than did rats exposed to 10 ppm, but this was the only significant dose-related change in excretion (see Table 10-1) (McKenna et al., 1978a). After intragastric administration of 50 mg/kg $^{14}$C-vinylidene chloride, fasted male rats exhaled a greater percentage of the dose as unchanged vinylidene chloride and a lesser percentage in the urine than did fed male rats (McKenna et al., 1978b). No such difference was apparent with a dose of 1 mg/kg. These results indicate a reduced capacity for metabolism

## TABLE 10-8

### Excretion of Radioactivity by Male Rats Given 0.5 mg/kg or 350 mg/kg $^{14}$C-Vinylidene Chloride Intragastrically, Intravenously, or Intraperitoneally [a]

| Size of Dose | Time (h) | Radioactivity Excreted (Percent of Dose)[b] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Intragastric | | | | Intravenous | | | | Intraperitoneal | | | |
| | | Exhaled Air | | | | Exhaled Air | | | | Exhaled Air | | | |
| | | Vinylidene Chloride | Carbon Dioxide | Urine | Feces | Vinylidene Chloride | Carbon Dioxide | Urine | Feces | Vinylidene Chloride | Carbon Dioxide | Urine | Feces |
| 0.5 mg/kg | 0-24 | 0.6±0.2 | 3.9±0.7 | 71.3±1.2 | 5.1±0.7 | 80.0±4.0 | 3.5±0.6 | 14.4±3.6 | 0.3 | 11.4±2.8 | 2.6±0.7 | 65.8±2.5 | 14.2±4.4 |
| | 24-48 | 0.06 | 0.5±0.4 | 5.3±0.9 | 2.7±0.9 | 0 | 0 | 0.7±0.2 | 0.1 | 0.2 | 0.5±0.1 | 2.0±0.1 | 1.6±1.1 |
| | 48-72 | 0.08 | 0.5±0.2 | 3.6±1.7 | 0.6±0.2 | 0 | 0 | 0 | 0 | 0.1 | 0.5±0.2 | 1.2±0.3 | 0.4 |
| | Total | 0.7±0.1 | 4.8±1.3 | 80.2±1.4 | 8.3±0.1 | 80.0±4.0 | 3.5±4.0 | 15.0±3.9 | 0.4 | 11.7±2.8 | 3.6±0.7 | 69.0±2.8 | 16.2±4.5 |
| 150 mg/kg | 0-24 | 62.4±4.3 | 0.3 | 17.6±4.4 | 0.4 | | | | | 90.5±2.9 | 0.7±0.4 | 7.1±2.1 | 0.5±0.2 |
| | 24-48 | 4.8±2.8 | 0.4 | 10.0±4.0 | 0.5±0.3 | | | | | 0.6±0.3 | 0.5±0.2 | 0.3 | 0.1 |
| | 48-72 | 0.1 | 0.3 | 1.9±1.0 | 0.4 | | | | | 0 | 0.1 | 0.3 | 0.1 |
| | Total | 67.3±4.3 | 29.5±6.7 | 1.3±0.4 | | | | | | 91.1±3.2 | 1.3±0.6 | 7.7±2.1 | 0.7±0.3 |

---

[a] Source: Jones and Hathway, 1978a

[b] Values shown are the Mean ± Standard Deviation of those means (n=four per group).

of vinylidene chloride in fasted animals. Fed/fasted and species differences in excretion that have already been noted in Section 10.1.2 will not be discussed here.

Following intragastric administration of 350 mg/kg $^{14}C$-vinylidene chloride, urinary excretion of radioactivity in intact male rats was approximately equal to the sum of the separate urinary and biliary excretions in male rats with biliary fistulae (Jones and Hathway, 1978a). This finding suggests that the origin of part of the urinary radioactivity was via the enterohepatic cycle.

Reported half lives of excretion for different nutritional states (fed versus fasted) and dose levels are compiled in Table 10-9. The only difference between fed and fasted rats was that fed rats excreted urinary metabolites more rapidly during the first 48 hours after administration of 200 ppm than did fasted rats (McKenna et al., 1978b). Changes in half-lives with increasing doses are inconsistent. It is not known whether the inconsistencies could be due to normal experimental variation, to an effect on more than one process, or to possible toxic effects.

10.1.4. Summary of Pharmacokinetics. Vinylidene chloride is readily absorbed by mammals following oral or inhalation exposure. Judging from excretion in the exhaled air and urine, most of an orally administered dose is absorbed through the gastrointestinal tract. Data for retention (as a percent of the inhaled concentration) during inhalation exposure were not encountered. Vinylidene chloride is metabolized in the liver with a number of possible reactive intermediates, including an epoxide, being formed. These reactive intermediates may react with macromolecules, producing toxic effects, and are detoxified primarily by conjugation with GSH. Excretion of metabolites and parent compound occurs primarily via the urine and the exhaled air, with greater percentages of the dose being exhaled as unchanged vinylidene chloride at high doses/exposures. Vinylidene chloride does not appear to be stored or accumulated in the tissues.

TABLE 10-9

Half-Lives of Excretion of $^{14}$C-Vinylidene Chloride, $^{14}CO_2$, and Radiolabeled Urinary Metabolites of $^{14}$C-Vinylidene Chloride

| | Dose | | Rates | | Half-lives | | |
|---|---|---|---|---|---|---|---|
| Excretion | Route | Amount | Sex | Nutritional Status | Rapid Phase | Slow Phase | Reference |
| **Pulmonary** | | | | | | | |
| Vinylidene chloride | intragastric | 1 mg/kg | male | fed and fasted* | 25 min | 117 min | McKenna et al., 1978b |
| | | 50 mg/kg | male | fed and fasted* | 21 min | 66 min | |
| | intragastric | 0.5 mg/kg | female | fed | 43 min | --- | Reichert et al., 1979 |
| | | 5.0 mg/kg | female | fed | 18 min | | |
| | | 50.0 mg/kg | female | fed | 27 min | 381 min | |
| | inhalation | 10 ppm | male | fed and fasted* | 20 min | 217 min | McKenna et al., 1978a |
| | | 200 ppm | male | fed and fasted* | 21 min | 133 min | |
| Carbon Dioxide | intragastric | 0.5 mg/kg | female | fed | 4 hr 30 min | 35 hr | Reichert et al., 1979 |
| | | 5.0 mg/kg | female | fed | 4 hr 45 min | 24 hr | |
| | | 50.0 mg/kg | female | fed | 2 hr 45 min | 20 hr 45 min | |
| **Urinary** | | | | | | | |
| Metabolites | intragastric | 1 mg/kg | male | fed and fasted* | 6 hr | 16.8 hr | McKenna et al., 1978b |
| | | 50 mg/kg | male | fed and fasted* | 6 hr | 16.8 hr | |
| | intragastric | 0.5 mg/kg | female | fed | 4 hr 30 min | 18 hr 30 min | Reichert et al., 1979 |
| | | 5.0 mg/kg | female | fed | 3 hr 45 min | 14 hr | |
| | | 50.0 mg/kg | female | fed | 5 hr 15 min | 25 hr | |
| | inhalation | 10 ppm | male | fed and fasted* | 3.1 hr | 19.3 hr | McKenna et al., 1978a |
| | | 200 ppm | male | fed | 4.5 hr | 1 hr | |
| | | 200 ppm | male | fasted | 3.8 hr | 23.9 hr | |

*One group fed and another group fasted

## 10.2 ACUTE, SUBACUTE, AND CHRONIC TOXICITY

### 10.2.1 Acute Exposure

As a class, the halogenated olefins and the chloroethylenes in particular show many similarities in their biologic effects. Anesthesia, hepatotoxicity, and nephrotoxicity are commonly measured indices of acute exposure in experimental animals, although considerable differences exist in relative potency. Factors that influence the toxicity of chloroethylenes include inherent chemical reactivity, species, sex, diet, and exposure to exogenous chemicals that modify drug-metabolizing enzyme activity. With the chloroethylenes (e.g., vinylidene chloride, trichloroethylene, vinyl chloride, perchloroethylene), toxic effects appear to be mediated by the metabolic formation of a critical intermediate, which is probably an epoxide (Andersen and Jenkins, 1977; Andersen et al., 1979b; Reynolds and Moslen, 1977; Jaeger, 1977).

Carpenter and coworkers (1949) published the first report on the toxicity of vinylidene chloride. They indicated that a single 4-hour inhalation exposure to 32,000 ppm was lethal to two, three, or four rats out of a group of six (exact number that died was not specified) over a 14-day observation period. More subjective information on the acute inhalation toxicity of vinylidene chloride was published by Irish (1962) at the Dow Chemical Company. He reported that exposures to 4000 ppm could rapidly produce stupor and unconsciousness, but noted that complete recovery from the anesthetic effect was probable if duration of exposure was brief. No-effect levels for experimental animals were estimated at 1000 ppm for up to 1 hour and 200 ppm for up to 8 hours. These values are in reasonable agreement with the data of Siegel et al. (1971), indicating that the 4-hour $LC_{50}$ for vinylidene chloride in rats is about 6350 ppm.

Over the past decade, several groups of investigators have explored the biochemical mechanism of vinylidene chloride-induced hepatotoxicity in rats.

These studies were prompted by early observations that the toxicity of vinylidene chloride was similar to that of carbon tetrachloride, a well-documented hepatotoxin (Jenkins et al., 1972). Initial studies involving the administration of single oral doses (500 mg/kg) to rats established that vinylidene chloride produced biochemical changes that were qualitatively similar to those produced by carbon tetrachloride (Jenkins et al., 1972). These included depressed liver glucose-6-phosphatase activity and increased activities of liver alkaline phosphatase and tyrosine transaminase, and of plasma alkaline phosphatase and alanine aminotransferase. In addition, vinylidene chloride displayed greater potency than did carbon tetrachloride in its effect on several of these biochemical parameters. In contrast to carbon tetrachloride, however, the biochemical alterations induced by vinylidene chloride were less pronounced in female rats and were reduced by pretreatment of animals with phenobarbital, an inducer of hepatic drug-metabolizing enzymes. On the other hand, Carlson and Fuller (1972) demonstrated that enzyme inducers (phenobarbital and 3-methylcholanthrene) as well as enzyme inhibitors (SKF 525A and Lilly 18947) increased the lethality of vinylidene chloride by inhalation in rats, whereas the opposite effect was seen in rats receiving carbon tetrachloride. These results strongly suggested a mechanism of toxic action for vinylidene chloride that differed from that for carbon tetrachloride. Confirmation of this belief was provided by Jaeger and coworkers (1973a,b), when they demonstrated that the hepatotoxic effects of vinylidene chloride are not due to a lipoperoxidative mechanism of action, as is the case with carbon tetrachloride.

Further studies on the hepatotoxicity of vinylidene chloride have focused on the relationship of metabolism to acute toxicity, and the influence of nutritional status, age, and sex on mortality. Jaeger and coworkers (1973a) observed that the acute lethality of vinylidene chloride administered by inhalation in male rats was greater during the period from 6:00 p.m. to 10:00 p.m. than during the period 6:00 a.m. to 10:00 a.m. This pattern of sensitivity to vinylidene chloride was correlated with the diurnal variation in hepatic GSH concentration, which is highest during the day and lowest during the night. Thus, the hypothesis was offered that a GSH-dependent pathway mediates the detoxification of vinylidene chloride, a mechanism which is known to be important in the detoxification of certain carcinogenic chemical intermediates (e.g., epoxides) (Jaeger, 1979). The importance of adequate hepatic GSH concentrations in protecting against acute intoxication with vinylidene chloride has subsequently been confirmed in studies using fasted animals that have reduced hepatic GSH levels (Jaeger et al., 1974; Andersen and Jenkins, 1977). In male rats fasted for 18 hours, the 24-hour $LC_{50}$ following a 4-hour inhalation exposure to vinylidene chloride was 600 ppm, whereas in fed animals an estimated $LC_{50}$ of 15,000 ppm was obtained (Jaeger et al., 1974). The minimum lethal concentration of vinylidene chloride was 200 ppm in fasted animals and 10,000 ppm for fed animals. The functional state of the thyroid gland, which apparently played a role in the regulation of GSH levels in the liver, also influenced the hepatotoxicity of vinylidene chloride (Jaeger et al., 1977b; Szabo et al., 1977). Chemical or surgical thyroidectomy was associated with an increase in hepatic GSH and a concomitant decrease in the mortality and hepatic necrosis caused by a 4-hour inhalation exposure of fasted male rats to 2000 ppm vinylidene chloride.

The studies of Jaeger (1977) and his coworkers (Jaeger et al., 1977a,b) led to the hypothesis that the mechanism of toxicity for vinylidene chloride involved damage to hepatic cellular mitochondria. This postulation was supported by evidence showing biochemical alterations following exposure of fasted male rats to vinylidene chloride (200-500 ppm for 1-24 hours); these alterations were indicative of an inhibition of the tricarboxylic acid cycle. Among these changes was a significant elevation of hepatic citric acid concentration in fasted (i.e., GSH-deficient) rats after 12 hours of exposure to 250 ppm vinylidene chloride in air. Thus, the authors suggested that vinylidene chloride may induce mitochondria-specific injury via the metabolic formation of monochloroacetic acid, which may subsequently give rise to monochlorocitric acid.

Studies conducted by Reynolds et al. (1975) and Reynolds and Moslen (1977) supported the theory that vinylidene chloride is a unique hepatotoxin in terms of its rapid action and its effect on mitochondria. The acute hepatotoxicity of chloroethylenes was shown to be, in decreasing order: vinylidene chloride > trichloroethylene > vinyl chloride > perchloroethylene. Exposure of rats to vinylidene chloride by inhalation (200 ppm, 4 hours) produced an abrupt hemorrhagic centrilobular necrosis. Parenchymal cell injury is characterized by retracted cell borders with pericellular spaces forming that may contain red blood cells and fibrin. Nuclear changes include the loss of perinucleolar chromatin and the aggregation of chromatin along the nuclear perimeter against the nuclear envelope. Within 2 hours after onset of exposure, swollen and ruptured mitochondria were observed in hepatic parenchymal cells, whereas the rough and the smooth endoplasmic reticulum appeared normal. In contrast, trichloroethylene, carbon tetrachloride, vinyl chloride, and perchloroethylene all produced damage to the endoplasmic reticulum.

Reynolds et al. (1980) associated the biochemical changes in the liver, such as sodium (Na), potassium (K), calcium (Ca), and GSH levels, with subsequent histological changes in rats exposed to 200 ppm vinylidene chloride for 1 to 4 hours. By the end of the first hour of exposure, sorbital dehydrogenase (SDH) activity was elevated and hepatic Na levels increased while GSH decreased. This trend continued for the second hour of exposure. By the third and fourth hour SDH, as well as serum alanine aminotransferase activity and Na and Ca levels, were greatly increased above controls. These increases remained high for 12 hours post-exposure. Histological injury included nuclear changes and centrilobular necrosis. Ion changes were attributed to changes in the membrane ion pumps, either directly or due to an adenosine triphosphate (ATP) deficiency. Decreased levels of GSH were attributed to a conjugation reaction in the metabolism of vinylidene chloride. Enzymatic activity increased during detoxification (Reynolds et al., 1980).

Similarly, Chieco et al. (1982) combined histological and chemical analyses to study the hepatotoxic effect of acute exposure to vinylidene chloride in rats. Histochemical testing was conducted at 1, 2, 4, or 6 hours after oral doses of 50, 100, 150, or 200 mg vinylidene chloride. The degree of liver or hepatocellular damage was assessed by histology and by measurements of liver ion levels (Na, Ca, and K) and serum transaminase activity. Injury was noted in the form of increased transaminase activity, decreased membrane adenosine triphosphatase activity, decreased succinate dehydrogenase activity (on the inner mitrochondrial membrane), and corresponding changes in the plasma and mitochondrial membrane histology. The investigators (Chieco et al., 1982) concluded that membranous organelles and membrane-bound enzymes were the primary sites of hepatocellular damage by vinylidene chloride. These changes in turn resulted in secondary alterations in glutathione levels, cellular ion imbalances, and cytoplasmic changes (Chieco et al., 1982).

Recently, vinylidene chloride has been shown to inhibit the activity of rat liver microsomal calcium pumps in the presence of a NADPH-generating system. The calcium pumps were not inhibited when vinylidene chloride was applied *in vitro* in the absence of a NADPH-generating system (Moore, 1980; Ray and Moore, 1982). Moore (1982) also reported inhibition of the liver endoplasmic reticulum calcium pump activity by vinylidene chloride. The concomitant rise in liver calcium levels occurred soon after vinylidene chloride administration and was attributed to the inactivity of the calcium pumps. Calcium released from the endoplasmic reticulum may serve as a trigger for the influx of extracellular calcium and, ultimately, cytotoxicity (Moore, 1982).

Information regarding the acute toxicity of vinylidene chloride is not restricted entirely to its effect on the liver. Jenkins and Andersen (1978) recently reported a nephrotoxic action of vinylidene chloride following oral administration to rats. Biochemical indices of kidney damage (plasma urea nitrogen, plasma creatinine) were elevated in fasted male rats given a single oral 400 mg/kg dose of vinylidene chloride (Table 10-10). Conversely, rats that were not fasted were protected from the nephrotoxic effect. At doses below 400 mg/kg, no significant alterations were noted in plasma indicators of kidney damage (Table 10-11). In addition, female rats were much less sensitive to the effects of vinylidene chloride on the kidney. In both male and female rats, however, histological evidence of renal damage was obtained at various times following the administration of a single oral dose of 400 mg/kg vinylidene chloride (Table 10-12). Since female rats showed the same, if not greater, histologic damage to the kidney as did males, it was suggested that biochemical parameters may not be sensitive indicators of kidney damage in females.

A single report has been published (Siletchnik and Carlson, 1974) concerning the cardiac sensitizing effects of vinylidene chloride by acute inhalation exposure in rats. Cardiac arrhythmias could not be produced by a single 4 μg/kg injection of epinephrine to male rats; however, when rats inhaled 25,600 ppm vinylidene chloride for 80 minutes, doses of epinephrine as low as 0.5 μg/kg could elicit serious cardiac arrhythmias, which could be further enhanced by pretreatment of rats with phenobarbital (50 mg/kg i.p. for 4 days).

## TABLE 10-10

### Influence of 24-Hour Fasting on the Effect of Oral Administration of Vinylidene Chloride (400 mg/kg) in Corn Oil on Plasma Urea Nitrogen Concentration in Male Rats (341 ± 7 g)[a]

| Status | Challenge | Hours After Challenge | Urea Nitrogen (mg/100 ml) |
|---|---|---|---|
| Fasted | Corn Oil | 24 | 21 ± 1[b] |
| | | 48 | 20 ± 1 |
| | | 72 | 22 ± 1 |
| Fasted | Vinylidene chloride | 24 | 51 ± 16[c] |
| | | 48 | 82 ± 39[c] |
| | | 72 | 50 ± 3[c] |
| Fed | Corn Oil | 24 | 20 ± 2 |
| | | 48 | 21 ± 1 |
| | | 72 | 22 ± 1 |
| Fed | Vinylidene chloride | 24 | 19 ± 2 |
| | | 48 | 12 ± 2[d] |
| | | 72 | 15 ± 1[c] |

[a] Source: Jenkins and Andersen, 1978

[b] All data are expressed as mean ± SE of groups of 3 to 6 rats.

[c] Significantly higher ($p<0.05$) than fasted, corn oil-challenged controls at the same time after challenge (method of analysis not mentioned).

[d] Significantly lower ($p<0.05$) than fed, corn oil-challenged controls at the same time after challenge.

## TABLE 10-11

### Relationships of Plasma Indicators of Kidney Damage to Dose 24 Hours After Oral Administration of Vinylidene Chloride to Male Rats (289 ± 9 g)[a]

| Dose (mg/kg) | Urea Nitrogen (mg/100 ml) | Creatinine (mg/100 ml) |
|---|---|---|
| 0 | 20 ± 2[b] | 0.76 ± 0.06 |
| 50 | 23 ± 1 | 0.61 ± 0.11 |
| 100 | 26 ± 1 | 0.62 ± 0.06 |
| 200 | 32 ± 7 | 0.80 ± 0.06 |
| 400 | 80 ± 1[c] | 2.33 ± 0.05[c] |
| 800 | 108 ± 18[c] | ND[d] |

[a] Source: Jenkins and Andersen, 1978

[b] All data are expressed as mean ± SE of groups of two to six rats.

[c] Significantly different from zero-dose control ($p<0.05$); however, the method of analysis was not mentioned.

[d] Not done

## TABLE 10-12

Comparison of Prevalence of Histopathologic Effects of Oral Administration of Vinylidene Chloride (400 mg/kg) in Male and Female Rat Kidneys[a]

| Time in hours after Vinylidene Chloride Treatment | Sex | Prevalence of Effect | | | | | |
|---|---|---|---|---|---|---|---|
| | | Normal | Chronic Inflammation[b] | Pigment | Dilitation[d] | Tubular Necrosis[e] | Vacuolization[f] |
| 0 | M | 6/8[g] | 1/8 | 1/8 | -- | -- | -- |
| | F | 2/4 | 2/4 | -- | -- | -- | -- |
| 2 | M | 7/8 | -- | -- | -- | -- | 1/8 |
| | F | 1/4 | 1/4 | 3/4 | -- | -- | -- |
| 4 | M | 8/8 | -- | -- | -- | -- | -- |
| | F | 1/4 | -- | 3/4 | -- | -- | -- |
| 8 | M | 4/4 | -- | -- | -- | -- | -- |
| | F | -- | -- | 4/4 | -- | -- | -- |
| 24 | M | 1/2 | -- | -- | 1/2 | -- | -- |
| | F | 1/4 | -- | 3/4 | 1/4 | -- | -- |
| 48 | M | 1/3 | -- | 2/3 | 2/3 | 1/3 | -- |
| | F | -- | -- | 2/4 | -- | 3/4 | 1/4 |
| 72 | M | -- | -- | 2/3 | 3/3 | 1/3 | -- |
| | F | -- | -- | 3/4 | 3/4 | 4/4 | 1/4 |

## TABLE 10-12 (Cont.)

| Time in hours after Vinylidene Chloride Treatment | Sex | Prevalence of Effect | | | | | |
|---|---|---|---|---|---|---|---|
| | | Normal | Chronic Inflammation[b] | Pigment | Dilitation[d] | Tubular Necrosis[e] | Vacuolization[f] |
| 96 | M | -- | -- | 2/3 | 1/2 | -- | -- |
| | F | 1/4 | -- | 2/4 | 1/4 | 1/4 | 1/4 |
| 120 | M | -- | -- | 2/2 | 1/2 | -- | -- |
| | F | -- | -- | 2/4 | 1/4 | 1/4 | 1/4 |
| 144 | M | -- | -- | 2/2 | 1/2 | -- | -- |
| | F | -- | -- | 3/4 | 4/4 | -- | -- |

---

[a] Source: Jenkins and Andersen, 1978

[b] Focal collections of mononuclear cells were present.

[c] Few to several tubules contained blue-black amorphous material.

[d] Focally, the tubules were distended and lined by basophilic regenerated epithelium.

[e] The tubular epithelium, chiefly proximal, showed eosinophilic coagulative necrosis with loss of cellular detail and inflammatory cell infiltration.

[f] The lining epithelium of occasional tubules contained cytoplasmic vacuoles.

[g] Number of tissues in which effect was seen per number of tissues examined.

Pulmonary damage has been observed in mice after either oral or i.p. administration of vinylidene chloride (Forkert and Reynolds, 1982; Krijgsheld et al., 1983). At 6 hours after administration, a single oral dose of 100 mg/kg produced degeneration of the endoplasmic reticulum of the Clara cells of the bronchiolar epithelium; a single oral dose of 200 mg/kg resulted in necrosis of both ciliated and Clara cells, with exfoliation of the bronchiolar epithelium (Forkert and Reynolds, 1982). Hypoxia (measured as a decrease in oxygen partial pressure of arterial blood) was observed 24 hours after administration of the higher dose, but the animals recovered and the bronchiolar epithelium had regained a normal appearance by 7 days. Clinical chemistry indices of liver damage [serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels] were greatly elevated even at the 100 mg/kg dose, 24 hours after administration.

Twenty-four hours after i.p. injection of mice with vinylidene chloride at a dose of 125 mg/kg, microscopic examination of the lungs revealed necrosis and sloughing of the Clara cells lining the bronchioles (Krigsheld et al., 1983). Pulmonary cytochrome P-450 levels and related monoxygenase activities were decreased; the authors suggested that these decreases may have been a reflection of the destruction of the Clara cells, which are known to contain relatively high levels of cytochrome P-450. The livers and kidneys of the treated mice were relatively unaffected, as judged by microscopic appearance, cytochrome P-450 content and monoxygenase activities.

### 10.2.1.1 Mechanisms of Toxicity

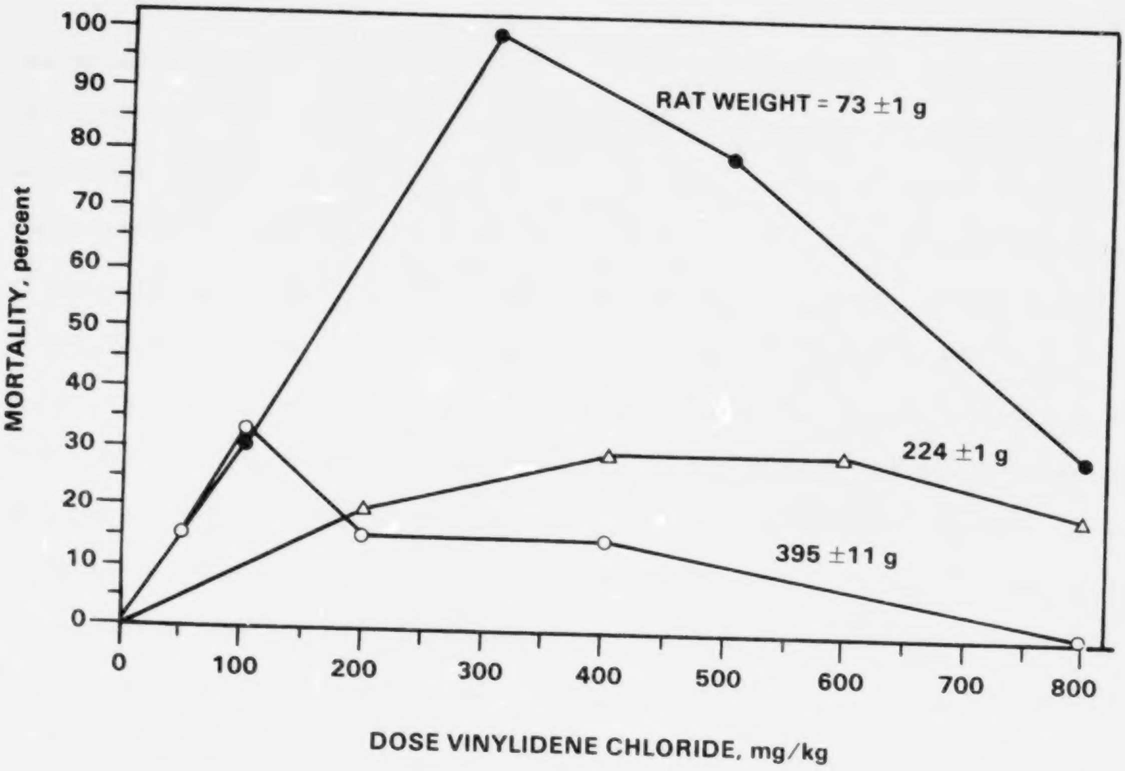
The acute toxicity of vinylidene chloride is altered by several factors in addition to the effects on sensitivity produced by fasting. A series of time-course and dose-response studies that explored mechanisms of toxicity for vinylidene chloride have recently been published.

Chieco et al. (1981) examined the effect of the administration vehicle on the toxicity and biological fate of vinylidene chloride in fasted and fed male Sprague-Dawley rats. Vinylidene chloride was given at 200 mg/kg orally in corn oil, mineral oil, or aqueous Tween-80 (0.5%; Sigma). Measurements of exhaled vinylidene chloride (unchanged) were taken at 15-minute intervals for 5 hours and rats were sacrificed at 6 hours for biochemical analyses as an assessment of hepatic injury. The vehicle did not affect the overall amount of vinylidene chloride exhaled, which ranged from 37 to 51% of the administered dose. The nature of the administration vehicle did, however, alter the rate at which vinylidene chloride was absorbed and exhaled. Aqueous Tween-80, which is readily absorbed, allowed the vinylidene chloride to be readily absorbed and exhaled in a shorter period of time. Corn oil was intermediate, while the poorly digested and absorbed mineral oil prolonged absorption and exhalation times in fasted and fed rats. Hepatic injury, measured as elevated SGOT and SGPT levels, was greatest in fasted rats treated with vinylidene chloride in mineral oil or corn oil (up to 150-fold increase in SGOT and SGPT levels). Fasted rats treated with vinylidene chloride in Tween-80 were moderately affected (15 to 18 times increase in SGOT and SGPT levels). In fed rats treated with vinylidene chloride in corn or mineral oil, enzymatic activity was slightly elevated (2 to 5 times) above the enzymatic activity in fed rats, while SGOT was not different from control levels. Chieco et al. (1981) suggested that the results of this study indicate that decreased hepatic injury in fed animals is due to the ability of these animals to detoxify vinylidene chloride over a longer period of time than fasted animals.

Histologically, fasted rats fed vinylidene chloride in corn or mineral oil showed massive hepatic necrosis, which was consistent with biochemical changes. Only scattered necrotic hepatocytes were seen in fasted animals given vinylidene chloride in aqueous Tween-80. Fasted animals given vinylidene chloride in corn oil or mineral oil also had granular "heme" casts in Henle's loop of the kidney. Concomitant high levels of free plasma hemoglobin and "pink urine" were noted in these animals. No pathological abnormalities were observed in the heart, lungs, spleen, adrenals, or duodenum in vinylidene chloride-treated animals (Chieco et al., 1981).

Andersen and Jenkins (1977) observed that body size or the age of the animal had a dramatic effect on the toxicity of vinylidene chloride when a single oral dose was given to fasted male rats. Dose-response curves for vinylidene chloride-induced mortality were constructed for animals of three different sizes (Figure 10-4). Mortality among large animals (395 ± 11 g) increased linearly from 0 to 35% at doses between 50 and 100 mg/kg; however, minimum lethal doses were found to be as low as 50 mg/kg. In medium-sized rats (224 ± 1 g), doses between 50 and 800 mg/kg produced mortality which varied from 10 to 33%. The most aberrant results were obtained in small rats (73 ± 1 g). Mortality increased to 100% at a dose of 300 mg/kg, but then decreased with increases in dose up to 800 mg/kg. The $LD_{50}$ values calculated from the data presented in Figure 10-4 would appear to be widely variable as shown by dependence on body size.

In an attempt to explain the effect of body size on acute mortality, Andersen and Jenkins (1977) examined the relationship between hepatotoxicity (indicated by plasma enzyme levels) and body size following the administration of a single oral 50 mg/kg dose of vinylidene chloride in corn oil. They found that both mortality within the first 24 hours and plasma enzyme activities after 24 hours were greatest among rats weighing between 130 and 160 g. In animals of this size, the estimated $LD_{50}$ was less than 50 mg/kg, whereas in large rats, the $LD_{50}$ would be 30 times greater. The unusual dose-mortality curve for immature rats (see Figure 10-4) was partially explained by the fact that increases in



○ Mortality in groups of 6 rats for 30 rats weighing 395 ±11 g.

● Mortality in groups of rats for 40 weanling rats weighing 73 ±1 g.

△ Mortality in groups of 10 rats for 40 rats weighing 224 ±1 g.

Figure 10-4. Dose-mortality curves for administration of single, oral doses of vinylidene chloride dissolved in corn oil to fasted male rats of various sizes.

Source: Andersen and Jenkins (1977a).

plasma transaminase activities paralleled mortality, and the retention of orally administered vinylidene chloride reached a maximum at a total dose of 100 mg/kg and then leveled off.

In comparison to fasted male rats, female animals were much less susceptible to the effects of vinylidene chloride. Plasma transaminase levels were not altered by a 50 mg/kg dose, regardless of body size. In female rats, the threshold dose for expression of acute toxic effects (plasma transaminase elevation) was about 100 mg/kg. Based on their results and on analogy to other halogenated hydrocarbons, Andersen and Jenkins (1977) postulated that vinylidene chloride is metabolized by a saturable microsomal enzyme-mediated reaction to a toxic intermediate that is probably an epoxide. A second enzyme-mediated reaction that converts the toxic intermediate to a less toxic form was also proposed. In young rats, this detoxification mechanism may be absent or present at only low levels, thus accounting for increased susceptibility.

In subsequent studies, Andersen and coworkers (1978, 1979b) examined in greater detail the relationship between vinylidene chloride metabolism and expression of toxic effects. Chemical modifiers of microsomal enzyme activity were administered to fasted male rats followed by a single oral dose of vinylidene chloride (Andersen et al., 1978). Phenobarbital pretreatment, which causes microsomal enzyme induction, protected rats against the lethal effects of vinylidene chloride. This protection by phenobarbital was opposite to that observed by Carlson and Fuller (1972) following inhalation exposure of rats. This protective action increased with increasing body size. Conversely, the microsomal enzyme inhibitor SKF 525A greatly enhanced the lethality of vinylidene chloride in large rats (261 ± 2 g) but had little influence in immature rats (88 ± 2 g). In addition, pretreatment of rats with the metabolic inhibitors pyrazole, aminotriazole, and carbon tetrachloride protected animals of all sizes against the lethal action of vinylidene chloride. These results supported the argument that two sequential enzyme-mediated reactions are involved in the activation of vinylidene chloride. The first reaction, which is inhibited by pyrazole, aminotriazole, and carbon tetrachloride, leads to formation of a toxic intermediate. The second reaction, which is affected by phenobarbital and SKF 525A pretreatment, is a detoxification step that apparently exists at greater levels in mature animals.

Subsequent studies by Andersen and coworkers (1979b) demonstrated that mortality induced in rats by inhalation of vinylidene chloride failed to follow a strict concentration x time relationship. Instead, mortality was more dependent on time of exposure than on concentration once a threshold level (about 200 ppm) had been reached (Figure 10-5). These data supported the involvement of a saturable enzymatic reaction in the production of a metabolite that is responsible for the expression of vinylidene chloride-induced toxicity. The authors appropriately noted that, under conditions of exposure that produce enzyme saturation, misleading estimates of the $LC_{50}$ (inhalation) or $LD_{50}$ (oral and parenteral) could result.

Studies by Short and coworkers (1977a,b) with rats and mice support the conclusions reached by Andersen et al. (1978, 1979b). Non-fasted animals (groups of 10) inhaled vinylidene chloride for 22 to 23 hours per day for up to 7 days. Short et al. (1977a,b) found that male mice were more sensitive than were male rats to lethal effects and hepatotoxic effects (measured by SGOT and SGPT activities) of vinylidene chloride (Table 10-13). Histopathologic examination revealed hepatic and renal damage among male mice exposed to 15, 30, and 60 ppm vinylidene chloride (1-5 days); hepatic damage only was seen in male rats exposed at 60 ppm for 3 days. When mice were given disulfuram (0.10% in feed 23 days before and during exposure), or thiram (0.10% in feed 3 days before and during

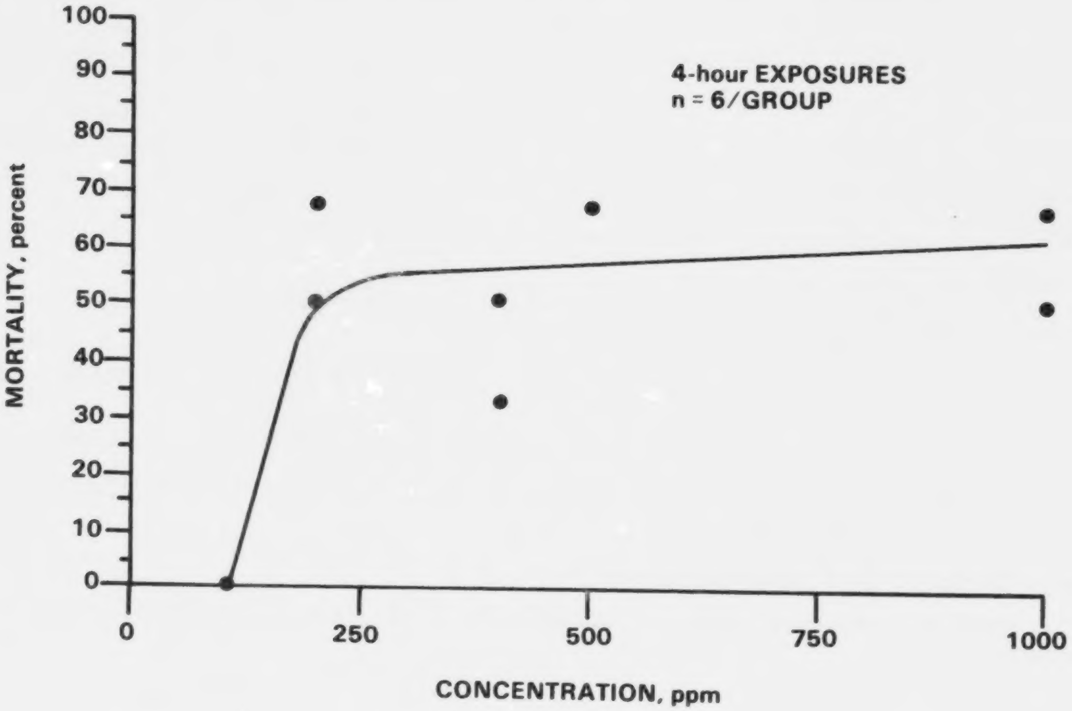


Figure 10-5. Effect of increasing concentration of vinylidene chloride on mortality in mature male rats. (Each point represents percentage mortality of a group of 6 animals exposed for 4 hours.)

Source: Andersen et al. (1978).

## TABLE 10-13

Toxicity of 60 ppm Vinylidene Chloride in Male Mice and Rats[a]

| Species | Days Exposed | SGOT (IU/l)[b] | SGPT (IU/l)[b] | Ratio Dead/Exposed |
|---|---|---|---|---|
| Mouse | 1 | 1946 ± 270 | 3045 ± 209 | 2/10 |
| | 2 | 751 ± 150 | 1112 ± 226 | 8/10 |
| Rat | 1 | 74 ± 6 | 44 ± 7 | 0/10 |
| | 2 | 263 ± 33 | 198 ± 29 | 0/10 |

[a] Source: Short et al., 1977b

[b] Mean ± SE for 2 to 5 determinations

SGOT = Serum glutamic-oxaloacetic transaminase; SGPT = Serum glutamic-pyruvic transaminase exposure), the acute lethality of vinylidene chloride was reduced. The authors observed that these compounds are known to protect against drug-induced toxicity, and may act by decreasing metabolic activation or increasing detoxification or both.

### 10.2.2 Subacute and Chronic Exposure

#### 10.2.2.1 Inhalation Studies

Several groups of investigators have characterized the toxicity of vinylidene chloride by repeated administration to experimental animals. Both ingestion and inhalation have been employed as routes of exposure. These studies established that, in general, the target organs affected by chronic exposures (i.e., liver and kidneys) are the same as those affected by acute administration of vinylidene chloride.

The first published report regarding the chronic toxicity of vinylidene chloride was a brief summary of inhalation studies conducted by the Dow Chemical Company (Irish, 1962). Unspecified animals exposed to 100 ppm and 50 ppm vinylidenechloride (5 days per week, 8 hours per day for several months) developed liver and kidney damage. Minimal lesions were also observed in the liver and kidney of animals exposed at concentrations of 25 ppm.

Prendergast and coworkers (1967) published the first detailed account of the chronic toxicity of vinylidene chloride. Groups of rats, rabbits, dogs, and monkeys were exposed in one of two ways: 30 exposures, 8 hours per day, 5 days per week to a concentration of 395 $mg/m^3$ (100 ppm) or 90 days of continuous exposure to a concentration of 189 $mg/m^3$ (48 ppm), 101 $mg/m^3$ (26 ppm), 61 $mg/m^3$ (16 ppm), or 20 $mg/m^3$ (5 ppm). The results of these studies are summarized in Table 10-14. The most prominent effects observed included hepatic damage in dogs, monkeys, and rats exposed to 189 $mg/m^3$; this damage consisted of fatty metamorphosis, focal necrosis, hemosiderin deposition, lymphocytic infiltration,

TABLE 10-14

Effect on Experimental Animals of Long-Term Inhalation of Vinylidene Chloride[a]

| Concentration | Schedule | Species | Mortality | Significant Findings |
|---|---|---|---|---|
| 100 ppm (395 ± 32 mg/m$^3$) | 30 exposures, 8 hr/day, 5 days/week | rat | 0/15 | None. |
| | | guinea pig | 0/15 | None. |
| | | rabbits | 0/3 | Weight loss in treated animals. |
| | | dog | 0/2 | None. |
| | | monkey | 0/3 | Weight loss in treated animals. |
| 48 ppm (189 ± 6.2 mg/m$^3$) | 90 days, 24 hr/day | rat | 0/15 | Animals gained less weight than controls. Hepatic lesions (see text). Renal lesions (see text). |
| | | guinea pig | 7/15 | Mortality occurred between day 4 and day 9 of exposure. Slight elevation of liver alkaline phosphatase activity and SGPT activity. |
| | | dog | 0/2 | Animals lost weight. Hepatic lesions (see text). One dog developed an adrenal cortical adenoma. |
| | | monkey | 3/9 | Mortality occurred on days 26, 60, and 64. Animals lost weight. Hepatic lesions (see text). |

## TABLE 10-14 (Cont.)

| Concentration | Schedule | Species | Mortality | Significant Findings |
|---|---|---|---|---|
| 26 ppm<br>(101 ± 4.4 mg/m$^3$) | 90 days,<br>24 hr/day | rat | 0/15 | None. |
| | | guinea pig | 3/15 | Mortality occurred between day 3 and day 5 of exposure. |
| | | rabbit | 0/3 | Animals lost weight. |
| | | dog | 0/2 | Animals lost weight. |
| | | monkey | 2/3 | Mortality occurred between day 3 and day 6 of exposure. |
| 16 ppm<br>(61 ± 5.7 mg/m$^3$) | 90 days,<br>24 hr/day | rat | 0/15 | Animals gained less weight than controls. |
| | | guinea pig | 3/15 | Mortality occurred on day 3 and day 4. |
| | | dog | 0/2 | None. |
| | | monkey | 0/9 | Animals lost weight. |
| 5 ppm<br>(20 ± 2.1 mg/m$^3$) | 90 days,<br>24 hr/day | rat | 2/45 | Animals gained less weight than controls. |
| | | guinea pig | 2/45 | None. |
| | | dog | 0/6 | Animals lost weight. |
| | | monkey | 1/21 | None. |
| Control | | rat | 7/304 | |
| | | guinea pig | 2/314 | |
| | | rabbit | 2/48 | |
| | | dog | 0/34 | |
| | | monkey | 1/57 | |

[a] Source: Prendergast et al., 1967

bile duct proliferation, fibrosis, and pseudo-lobule formation. Rats also showed nuclear hypertrophy of the renal tubular epithelium when exposed to vinylidene chloride at 189 mg/m$^3$.

Gage (1970) conducted limited studies in rats exposed to vinylidene chloride by inhalation that support the previous observations of Prendergast and coworkers (1967). Four male and four female rats received twenty 6-hour exposures to vinylidene chloride at concentrations of 500 or 200 ppm. At the higher concentration, retarded weight gain and nasal irritation were noted. Liver cell degeneration was observed upon autopsy. Inhalation of 200 ppm vinylidene chloride produced slight nasal irritation but no significant findings at autopsy; however, the group size was very small.

Lee and coworkers (1977) exposed CD-1 mice (36 males and 36 females) to 55 ppm of vinylidene chloride for 6 hours per day, 5 days per week, for up to 12 months. Two male mice died on the 13th day of exposure, and revealed acute toxic hepatitis and tubular necrosis of the renal cortex. Hepatic hemangiosarcomas were observed in three exposed mice, and various hepatic lesions were generally observed that included the following: enlarged and basophilic hepatocytes, enlarged nuclei with eosinophilic inclusions, mitotic figures, or polyploidy, microfoci of mononuclear cells, focal degeneration, and necrosis. Specific incidences were not given. Hepatic hemangiosarcomas were not observed in any of 72 control mice. Bronchiolo-alveolar adenomas were observed in six exposed mice and one control mouse.

Dow Chemical Company performed an inhalation toxicity study with vinylidene chloride and interim results were published by Rampy and coworkers (1977). In a 90-day study, groups of 40 Sprague-Dawley rats (20 male and 20 female) were exposed for 6 hours per day, 5 days per week, to vinylidene chloride at a concentration of 25 or 75 ppm. Eight animals of each sex were sacrificed after

30 days, and the remainder killed after 90 days. Among animals exposed at both concentrations, increased cytoplasmic vacuolation of hepatocytes was seen 30 days or longer after initial exposure. No other remarkable effects were attributed to the vinylidene chloride exposure.

In another study, Rampy and coworkers (1977) exposed Sprague-Dawley rats for 18 months (6 hours/day, 5 days/week) to vinylidene chloride at several concentrations in air. For the first 5 weeks, rats (104 males and 104 females) were exposed to levels of 10 or 40 ppm, after which the concentrations were raised to 25 and 75 ppm, respectively, for the remainder of the 18-month exposure period. Animals were maintained for an additional 6 months after termination of exposure. Although male rats gained weight at a slightly slower rate than controls, no clinical evidence of overt toxicity was seen during the study. Both male and female rats sacrificed at an interim of 1 year, however, showed hepatic lesions consisting of increased cytoplasmic vacuolation of hepatocytes. In addition, higher kidney weights were seen in female rats exposed at both treatment levels of vinylidene chloride.

The final data of this 2-year inhalation study (interim results reported by Rampy et al., 1977) were recently compiled and reported by McKenna et al. (1982). Data were reported on hematologic parameters, urinalysis, clinical chemistry, cytogenic changes in the bone marrow, body weight, major organ weights, and histopathology following necropsy on all animals (either at interim periods of 1, 6, 12, or 18 months or at the termination of the experiment at 734 to 736 days). Statistical evaluations of data were made using analysis of variance and Dunnett's test (body weight, hematology, urinalysis, clinical chemistry, and organ weight data) or by the Fischer exact test (mortality rates, gross and microscopic pathologies, and tumor incidence). The level of significance was chosen as $P \leq 0.05$.

Mortality figures for male rats exposed to vinylidene chloride at either dose level did not reveal significant increases. Females exposed at 25 ppm vinylidene chloride had increased mortality rates when compared to controls from the 15th through 22nd months. Statistically significant increased mortality rates in females exposed at 75 ppm vinylidene chloride were noted during months 15, 17, and 21 of the study. The earlier onset of mammary tumors and higher incidences of pneumonia in treated groups may have contributed to these higher mortality rates.

No consistent dose-related decrease in mean body weight for male rats exposed to 25 or 75 ppm vinylidene chloride was observed. A general trend of decreased body weight was noted in exposed males when compared to control males but increased mean body weights were noted in the exposed groups sporadically throughout the study. Generally, females exposed to either 25 or 75 ppm vinylidene chloride had higher mean body weights for the first 6 months but were comparable to controls throughout the remainder of the study.

No significant dose-related effects in hematological, urinalysis, clinical chemistry, or cytogenetic parameters were reported in any exposure group. The only significant changes in organ weight were a decreased mean liver weight in males exposed to 25 or 75 ppm vinylidene chloride at the 1-year interim sacrifice and an increased mean kidney weight in both exposure groups of female rats at 1 year. Both organ weight changes were statistically significant when compared to controls and considered to result from vinylidene chloride exposure (McKenna et al., 1982).

Many gross and microscopic lesions were seen in control and vinylidene chloride-exposed rats; most were spontaneously occurring, age-related changes common to Sprague-Dawley rats. The incidence of lesions from chronic murine pneumonia in exposed male and female rats was elevated, but lower levels in controls were attributed to the physical separation of exposed and control groups which deterred the spread of pneumonia. Chronic renal disease was the predominant cause of death in male rats of the study. Females had a high incidence of subcutaneous tumors of the mammary gland region which was frequently the cause of death. There was a statistically significant increase in the incidence of hepatocellular fatty change in female rats exposed to 75 ppm during the 18-month exposure period. These changes were reversed during the 6-month post-exposure period, however. Females treated at 25 ppm showed a similar but non-significant trend. This reversible and non-progressive liver change was noted in males at the 6 and 12 months interim sacrifice but not at 18 months.

The incidence of pituitary adenomas was decreased in both dose groups of male rats and the 25 ppm vinylidene chloride dose group of females. There were fewer pancreatic islet cell adenomas and thyroid adenocarcinomas in male rats exposed to 75 ppm vinylidene chloride. Mammary adenocarcinomas were statistically increased in female rats treated at 25 ppm vinylidene chloride. Subcutaneous or mammary tumors were more prevalent in 75 ppm vinylidene chloride treated females than in controls. The total number of rats with primary neoplasms was similar for both sexes at all dose groups, including controls. The total number of rats with primary neoplasms was similar (ranging from 84 to 86) for all six dose/sex groups. Summarizing the tumor incidence data, the authors reported that none of the statistically significant differences were considered to be direct effects of vinylidene chloride exposure.

### 10.2.2.2 Ingestion Studies

A single long-term study has been conducted with vinylidene chloride administered in the drinking water of rats. This study was conducted by the Dow Chemical Company for the Manufacturing Chemists' Association (Humiston et al., 1978). Groups of 96 Sprague-Dawley rats (48 males and 48 females) were exposed for 2 years to vinylidene chloride incorporated into the drinking water at nominal concentrations of 50, 100, and 200 ppm. These dose levels corresponded to approximate daily intakes of vinylidene chloride in the range of 5-12, 8-20, and 16-40 mg/kg at the 50, 100, and 200 ppm concentrations, respectively. In comparison to control animals, vinylidene chloride-treated rats displayed no significant or consistent differences in general appearance, body weight, food consumption, water consumption, hematologic values, urinalysis, clinical chemistry values, or organ weights. Gross and histopathologic examination of tissues from treated rats, however, revealed a number of statistically significant lesions. These are partially summarized in Table 10-15. The authors considered the most important lesions to be the hepatocellular fatty change and periportal hepatocellular hypertrophy which occurred in male rats at the 200 ppm dose level and in females at all dose levels. The authors did not observe any hepatocellular necrosis that could be considered treatment-related.

Results of this long-term, oral study with rats were subsequently published in the open literature by Quast et al. (1983), along with results of a 97-day oral study with dogs; the dog study was also conducted by the Dow Chemical Company. Groups of four male and four female beagle dogs were administered vinylidene chloride in doses of 0, 6.25, 12.5, or 25 mg/kg daily for 97 days. The chemical and vehicle (peanut oil) or vehicle alone was administered orally in a capsule once a day. As compared with controls, the groups of treated dogs had no differences in general appearance or demeanor, body weight, food consumption, hematologic and clinical chemistry values, urinalysis, organ weights and gross and microscopic appearance of the tissues.

### 10.2.3 Summary of Toxicity

The biologic activity of vinylidene chloride has been thoroughly studied in experimental mammals; no information is available concerning the effects of vinylidene chloride on domestic animals or wildlife.

## TABLE 10-15

## Pathologic Effects of Long-Term Ingestion of Vinylidene Chloride Incorporated in the Drinking Water of Sprague-Dawley Rats[a]

| Effect | Dose Level | | | | | |
|---|---|---|---|---|---|---|
| | 50 ppm | | 100 ppm | | 200 ppm | |
| | M | F | M | F | M | F |
| Increased incidence of intra-abdominal fluid or blood in the abdominal cavity | | | X | | | |
| Increased incidence in the total number of rats with hepatocellular fatty change or fatty degeneration | | | | X | X | X |
| Increased incidence of hepatocellular fatty change with location in lobule not specified | | X | | X | | X |
| Increased incidence in periportal hepatocellular fatty change | | | | | | X |
| Increased incidence of periportal hepatocellular hypertrophy | | X | | X | X | X |
| Increased incidence of hepatic centrilobular atrophy | | | | | | X |
| Increased incidence of mammary gland fibroadenomas/adenofibromas | | X | | | | |

[a] Source: Humiston et al., 1978

M = male; F = female

The toxicity of vinylidene chloride varies with the age, sex, and species of the animal exposed. In the most studied animal, rat, inhalation exposure to 32,000 to 6,350 ppm has proven to be fatal. In rats fasted prior to exposure, the $LC_{50}$ value was as low as 600 ppm. For oral adminstration of vinylidene chloride, however, the determination of an $LD_{50}$ value is difficult as a result of the relatively flat dose/mortality curve which possesses an extended plateau region. The target organs affected by acute exposure to vinylidene chloride are the liver and kidneys, with evidence from studies of the liver to indicate that mitochondrial damage occurs. Both liver and kidney damage are also observed following subchronic and chronic exposure to vinylidene chloride by either inhalation or ingestion. Continuous inhalation exposure of rats to 5 ppm of vinylidene chloride has been shown to adversely affect body weight gain while ingestion of vinylidene chloride by rats at 5 to 12 mg/kg/day produced fatty livers. A no-observed effect level (NOEL) for inhalation exposure (<5 ppm) or ingestion (<5 mg/kg/day) has not been demonstrated.

## 10.3 TERATOGENICITY AND REPRODUCTIVE TOXICITY

Short et al. (1977c) performed an extensive investigation of the toxicity during gestation of inhaled vinylidene chloride in mice and rats (approximately 20 animals in each of the exposed groups and 60 in the control groups). This study examined toxicity to the dams, toxicity to the fetus, and teratogenic and behavioral effects in the pup. In the initial part of the experiment, rats (18 to 20 per group) were exposed to 15, 57, 300, and 449 ppm vinylidene chloride, and mice to 15, 30, 57, 144, and 300 ppm vinylidene chloride for 23 hours per day from day 6 to 16 of gestation. In rats, appreciable deaths (over 25%) of the dams occurred in the 300 to 449 ppm group, and maternal well-being, as measured by food consumption and weight gain, was adversely affected in the 15 ppm and above groups. Similar results were observed in mice, with no pregnant mice surviving at the 144 or 300 ppm levels, and food consumption and weight gain being reduced in all groups except the 15 ppm animals. Early resorptions were common in exposed animals, with 100% resorption in mice exposed to 30 and 57 ppm vinylidene chloride (resorption in the 15 ppm groups was comparable to controls), and 49 and 64% resorption in rats exposed to 57 and 449 ppm vinylidene chloride, respectively. Dams that survived the exposure at other concentration levels had resorption rates comparable to those of controls. There were no increases in external gross abnormalities noted in surviving pups in any of the exposed groups of rats or mice; however, fetotoxicity was observed as soft tissue anomalies in rats (none were observed in mice) and some skeletal ossification problems in all groups of mice and rats. The authors concluded that these anomalies could not be evaluated in regard to teratogenic effects, since there were overt signs of toxicity (reduced weight gain and food consumption) due to the treatment in the dams. Abnormalities at a similar incidence to those observed in the treated animals were also observed in a group of animals on a food-restricted diet.

The study was continued using only mice, with groups of 10 to 24 dams exposed to a variety of concentrations of vinylidene chloride for different periods of time during gestation (Table 10-16), in an attempt to alleviate maternal toxicity as usually indicated by decreased maternal weight gain. By shortening the exposure periods, the incidence of resorption was lessened; however, treatment-related weight loss in the dams was still evident. It was interesting to note that exposures starting on days 10 and 12 and ending on day 15 were associated with a higher resorption rate than that observed when exposures were started on day 8 of gestation. This may have occurred because of some adaptation of the animals as a result of the longer exposure prior to a critical period during the latter part of gestation. Pups from vinylidene chloride- exposed dams again had a variety of soft tissue and skeletal anomalies,

### TABLE 10-16

### Exposure Levels and Duration of Exposure to Vinylidene Chloride During Gestation in Mice[a]

| Vinylidene Chloride ppm | Days of Gestation 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 54 | 6--- | --- | --- | --- | --- | --- | --- | --- | --- | 15 | | |
| 74 | | | 8--- | --- | --- | --- | --- | --- | --- | 15 | | |
| 54 | | | 8--- | --- | --- | --- | --- | --- | --- | 15 | | |
| 41 | | | 8--- | --- | --- | --- | --- | --- | --- | 15 | | |
| 54 | | | | | 10--- | --- | --- | --- | --- | 15 | | |
| 54 | | | | | | | 12--- | --- | --- | 15 | | |
| 112 | 6--- | --- | --- | 9 | | | | | | | | |
| 81 | 6--- | --- | --- | 9 | | | | | | | | |
| 56 | 6--- | --- | --- | 9 | | | | | | | | |
| 112 | | | | 9--- | --- | --- | 12 | | | | | |
| 81 | | | | 9--- | --- | --- | 12 | | | | | |
| 56 | | | | 9--- | --- | --- | 12 | | | | | |
| 112 | | | | | | | 12--- | --- | --- | 15 | | |
| 81 | | | | | | | 12--- | --- | --- | 15 | | |
| 56 | | | | | | | 12--- | --- | --- | 15 | | |
| 112 | | | | | | | | | | 15--- | --- | 17 |
| 81 | | | | | | | | | | 15--- | --- | 17 |
| 56 | | | | | | | | | | 15--- | --- | 17 |

[a] Source: Short et al., 1977c

---------- = Duration of Exposure

but these occurred only when maternal welfare had been adversely affected by the exposure to vinylidene chloride. In addition, the behavioral studies demonstrated no major adverse effect on pups exposed _in utero_ to vinylidene chloride. These tests included the following: surface righting, pivoting, auditory startle, bar-holding, righting in air, visual placing, swimming ability, physical maturation, and activity test. The authors concluded that vinylidene chloride was possibly a weak teratogen based on the increase in soft tissue anomalies although these anomalies were not statistically significant. However, a conclusion of teratogenicity is weakened by additional effects on the pups as a result of maternal toxicity at nearly all concentrations of vinylidene chloride used.

Murray et al. (1979) exposed rabbits to vinylidene chloride by inhalation, and rats by inhalation and by incorporating the compound into the drinking water (Table 10-17). Exposure occurred during days 6-15 or 6-18 of gestation for rats and rabbits, respectively. Inhalation exposure was for 7 hours/day to vinylidene chloride which was 99.5% pure and contained the polymerization inhibitor monomethylether of hydroquinone (MEHQ) at a level of 200 to 400 ppm. An exposure level of 200 ppm was employed in the drinking water study using redistilled vinylidene chloride containing only 1 to 5 ppm of residual MEHQ. Upon termination of the observation period, on days 21 and 29 of gestation for rats and rabbits, respectively, the dams were killed and examined for changes in liver weight, and in non-pregnant animals for signs of conception. The fetuses were measured, examined for external anomalies, and one-third of the animals were dissected for soft-tissue alterations. All fetuses were examined for skeletal alterations.

In the inhalation study in rats, maternal weight gain decreased in the 80 and 160 ppm groups during the exposure period and increased during the post-

## TABLE 10-17

### Number of Animals and Exposure Levels Used to Study the Teratogenicity of Vinylidene Chloride[a]

| Route | Concentration | Species | No. of Animals |
|---|---|---|---|
| inhalation | 160 | rats | 30 |
| | 160 | rabbits | 18 |
| | 80 | rats | 30 |
| | 80 | rabbits | 22 |
| | 20 | rats | 44 |
| | 0 | rats | 20-47/group |
| | 0 | rabbits | 16 |
| oral | 200 | rats | 26 |
| | 0 | rats | 24 |

[a] Source: Murray et al. (1979)

exposure period, while no changes (in comparison to controls) were observed in the 20 ppm groups or the animals in the drinking water study. Food consumption followed similar trends. Only in dams exposed to 160 ppm was there an increase in the liver to body weight ratio, although the absolute liver weight was similar to control animals. Exposure to vinylidene chloride did not affect the outcome of pregnancy as indicated by number of implants, live fetuses, or resorption rates, nor were there changes in fetal sex ratios or fetal body weights. There was a significant increase in the crown-rump length in fetuses of rats receiving vinylidene chloride in the drinking water. Although no increase in major malformations (taken individually or collectively) was present, the fetuses of animals exposed in the 80 and 160 ppm groups showed an increased incidence of minor skeletal alterations (Table 10-18). The most common skeletal alteration was wavy ribs, which was observed to be more severe at the higher exposure level. Wavy ribs and delayed ossification were interpreted as fetotoxic and embryotoxic manifestations of maternal toxicity at the higher exposure levels. The authors noted that the consumption by rats of drinking water containing 200 ppm of vinylidene chloride resulted in fewer adverse effects (dam and offspring) than did exposure to either 80 or 60 ppm of the test material by inhalation. The average daily dose of vinylidene chloride received by rats given the compound in drinking water was calculated to be approximately 40 mg/kg/day. Estimates of the total amount of vinylidene chloride by gavage is approximately equivalent to a single 7-hour inhalation exposure of 120 ppm. The sensitivity of the rat to vinylidene chloride was apparently greater when vinylidene chloride was inhaled than when it was ingested.

The authors speculated on a possible mechanism to account for route dependent differences in the toxicity of vinylidene chloride. Detoxification of vinylidene chloride has been reported to occur via conjugation of its active

## TABLE 10-18

### Incidence of Fetal Alterations Among Rats Exposed to Vinylidene Chloride By Inhalation or by Ingestion[a]

| | Route of exposure[b] (ppm vinylidene chloride) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Inhalation | | | | | | Drinking Water | |
| | 0 | 20 | 0 | 80 | 0 | 160 | 0 | 200 |
| | No. fetuses examined/No. litters examined | | | | | | | |
| External and skeletal examination | 470/40 | 462/40 | 230/20 | 292/27 | 209/17 | 291/26 | 250/24 | 290/25 |
| Soft tissue examination | 162/40 | 158/40 | 77/20 | 103/27 | 70/17 | 100/26 | 86/24 | 100/25 |
| Bones of the skull | 308/39 | 304/39 | 153/20 | 189/26 | 139/17 | 191/26 | 164/23 | 190/24 |
| | No. fetuses (litters) affected | | | | | | | |
| External examination | | | | | | | | |
| Multiple defects[c] | 1(1) | 0 | 0 | 0 | 1(1) | 0 | 0 | 0 |
| Omphalocele | 0 | 0 | 0 | 1(1) | 0 | 0 | 0 | 0 |
| Narrow head, point snout | 0 | 0 | 0 | 1(1) | 0 | 0 | 0 | 0 |
| Soft tissue examination | | | | | | | | |
| Diaphragmatic hernia | 0 | 0 | 1(1) | 0 | 0 | 0 | 0 | 0 |
| Ectopic ovaries (only) | 1(1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Skeletal examination | | | | | | | | |
| Missing one thoracic and one lumbar vertebrae | 0 | 0 | 0 | 0 | 2(1) | 14(4) | 0 | 0 |
| Extra thoracic vertebrae and pair of ribs | 0 | 0 | 0 | 0 | 1(1) | 0 | 0 | 0 |
| Vertebrae, delayed ossification of centra of cervical vertebrae[d] | 34(17) | 58(19) | 24(14) | 47(17) | 30(13) | 107(20)[e] | 46(16) | 72(18) |
| Wavy ribs[d] | | | | | | | | |
| Type I[f] | 5(5) | 10(7) | 0 | 9(8)[e] | 2(1) | 9(5) | 0 | 3(2) |
| Type II[f] | 4(4) | 3(2) | 0 | 2(2) | 1(1) | 13(9)[e] | 1(1) | 0 |
| Total | 9(7) | 13(7) | 0 | 11(9)[e] | 3(1) | 22(10)[e] | 1(1) | 3(2) |
| Skull, delayed ossification[d] | 42(17) | 36(18) | 13(7) | 38(13)[e] | 24(8) | 59(20)[e] | 19(10) | 32(11) |
| Total malformed fetuses | 2(2) | 0 | 1(1) | 2(2) | 4(2) | 14(4) | 0 | 0 |

[a] Source: Murray et al., 1979

[b] Rats were exposed to 20, 80, or 160 ppm vinylidene chloride for 7 hours/day or were given drinking water containing 200 ppm of the compound from days 6 to 15 of gestation.

[c] Each of these fetuses had: short trunk, hypoplastic tail, ectopic ovaries, missing all lumbar and sacral vertebrae, and missing ribs.

[d] This alteration was considered to be a skeletal variant and was not included in the calculation of the total malformed fetuses.

[e] Significantly different from control value by a modified Wilcoxon test, $p<0.05$

[f] Type 1, gentle wave; Type II, callous or "U"-shaped bend in rib

metabolite with glutathione (McKenna et al., 1977). Since the levels of glutathione have been reported to undergo diurnal variations the authors hypothesized that the available levels of glutathione might be high enough to prevent toxicity when vinylidene chloride was administered in drinking water but not when the animals were exposed via inhalation. The levels of glutathione would probably be expected to be low when the inhalation exposure was given (8:00 am - 3:30 pm) since gultathione levels are reported to peak in the evening and early morning (Jaeger et al., 1973; Watanabe et al., 1976). Vinylidene chloride in drinking water was made available to the rats over a 24 hour period. The authors offered this speculation in the discussion of experimental results but did not provide experimental data to support this hypothesis. Without actual experimental data, it is not possible to make any conclusions on this interpretation.

In rabbits exposed to 160 ppm of vinylidene choride, there was a decrease in body weight gain during exposure and an increase after termination of exposure. Only dams exposed at 80 ppm had an increase in liver to body weight ratio. There was also an increase in the resorption rate in the 160 ppm group, with resorption associated with animals having the greatest weight loss. Similar to the study in rats, exposure to vinylidene chloride produced no increase in major malformations, although there was an increase in minor skeletal alterations in the 160 ppm group (Table 10-19). Again, the fetal anomalies observed in rabbits were attributed to maternal toxicity rather than to a teratogenic effect of vinylidene chloride.

The effect of vinylidene chloride on reproduction and its teratogenic potential when ingested in the drinking water was tested in a three generation study in Sprague-Dawley rats (Nitschke et al., 1980; Nitschke et al., 1983). The parental generation ($f_o$), consisting of 10 male and 20 females in each of three

TABLE 10-19

Incidence of Fetal Malformation Among Litters of Rabbits Exposed to Vinylidene Chloride[a]

| | Vinylidene Chloride[b] (ppm) | | | |
|---|---|---|---|---|
| | 0 | 80 | 0 | 160 |
| | No. fetuses/No. litters examined | | | |
| External and skeletal examination | 116/14 | 155/18 | 111/13 | 91/12 |
| Soft tissue examination | 44/14 | 56/18 | 42/13 | 37/12 |
| External examination | No. fetuses (litters) affected | | | |
| No malformation observed | | | | |
| Soft tissue examination | | | | |
| Dilated cerebral ventricles | 0 | 1(1) | 0 | 0 |
| Thinning of ventricular wall of heart | 0 | 0 | 0 | 1(1) |
| Skeletal examination | | | | |
| Missing ribs | 0 | 0 | 1(1) | 0 |
| Extra vertebrae | 0 | 1(1) | 1(1) | 0 |
| Hemivertebrae and fused ribs | 0 | 1(1) | 0 | 1(1) |
| Total malformed fetuses | 0 | 3(3) | 2(2) | 2(2) |

---

[a] Source: Murray et al., 1979

[b] Rabbits were exposed to 80 or 160 ppm vinylidene chloride for 7 hr/day from days 6 to 15 of gestation. No value differed significantly from the control value by a modified Wilcoxon test, $p<0.05$.

treatment groups, received 50, 100, or 200 ppm vinylidene chloride. No vinylidene chloride was administered to a control group of 15 males and 30 females. Equivalent doses for males ingesting 50, 100, or 200 ppm vinylidene chloride continuously in the drinking water were 6, 10, or 19 mg/kg/day, and 8, 13, or 26 mg/kg/day, respectively. Following 100 days of exposure to vinylidene chloride, all groups of males and females ($f_o$) were mated (within dose groups), resulting in the first filial ($f_{1A}$) generation. Due to the low fertility rate during this initial breeding, the $f_o$ rats were re-mated after a period of no less than 10 days after weaning of the $f_{1A}$, which produced the $f_{1B}$ offspring. The $f_o$ generation rats were exposed to vinylidene chloride at the three respective doses for a 2-year toxicity study (Humiston et al., 1975). The $f_{1A}$ and $f_{1B}$ litters of all dose groups were examined for reproductive indices (number of litters/number of dams; survival of pups at 1, 7, 14, and 21 days). The animals to be raised as the $f_1$ generation adults were randomly selected from the $f_{1B}$ generation and continued to be exposed to vinylidene chloride. All other $f_{1A}$ and $f_{1B}$ rats were sacrificed at 21-24 days of age for internal and external examinations.

At ≃110 days of age, the $f_1$ adults were mated, producing the $f_2$ progeny. The $f_2$ litters were examined similar to the previous litters, and randomly selected males and females were maintained for $f_2$ adults. The remaining $f_2$ weanlings were terminated and examined for external and internal aberrations. Adult $f_2$ rats were mated at ≃110 days of age to produce the $f_{3A}$ gneration. Survival of the $f_{3A}$ litters in all groups receiving vinylidene chloride was decreased so the $f_2$ adults were re-mated after the $f_{3A}$ litters were weaned. To determine if the decreased survival in the dose groups was due to prenatal or postnatal exposure to vinylidene choride, one-third of the $f_{3B}$ litters born to 200 ppm vinylidene chloride-treated dams were exchanged with litters of untreated dams. No dose-related decreased survival was noted in any groups of the $f_{3B}$ litter. At least 10 days after the $f_{3B}$ litters were weaned, the $f_2$ parents were mated again to produce the $f_{3C}$ litters to determine survival rates of this group. A selected number of male and female rats from each dose group of the $f_{3B}$ litters were raised for 185-213 days as $f_3$ adults. All remaining weanling rats of the $f_{3A}$, $f_{3B}$, and $f_{3C}$ litters were sacrificed at 21-24 days of age for external and internal examination (Nitschke et al., 1980).

The water containing the vinylidene chloride was available continuously throughout this study to all treated rats. The first generation rats ($f_o$) were treated during their maturation, during mating periods, throughout gestation and lactation for females, and were subsequently used in a 2-year toxicity study. Pups were exposed prenatally and throughout their lives until their sacrifice, or longer if they were selected as breeders of that generation (Nitschke et al., 1980).

The results of this study indicate few, if any, dose-related effects on reproduction or the development of progeny in rats treated with vinylidene chloride in the drinking water at the dose levels tested (Nitschke et al., 1980). A slight decrease in the fertility (number of litters/number of mated dams) of the $f_o$ rats was noted in controls and the two highest dose groups (120 and 200 ppm) when compared to historical controls of Sprague-Dawley rats. Survival of $f_2$ progeny was decreased in all treated groups but this response was not dose-related since animals treated at 100 ppm vinylidene chloride showed the greatest mortality, while groups treated at 50 and 200 ppm were similar. Decreased survival during the lactation period in the $f_{3A}$ generation did appear dose-related, but survival in the $f_{3B}$ litters of dams given 100 and 200 ppm vinylidene chloride was higher than controls. It was not possible to ascertain the cause, whether it be prenatal or postnatal exposure to vinylidene chloride, of the decreased survival in the $f_{3A}$ generation mice. No decreased survival in any group of the $f_{3B}$, including those cross-fostered (control group dams rearing 200 ppm progeny and vice versa), was noted. The decreased survival of the $f_{3A}$ generation was negated since the similarly treated $f_{3C}$ generation showed no dose-related, decreased survival.

Fetal survival, litter size, and growth rates of the neonates showed no consistent dose-related effects for any generation during this study. At necropsy, histopathological examination revealed hepatocellular fatty change and pronounced hepatic lobular patterns in adult $f_1$ rats treated with 100 or 200 ppm vinylidene chloride. Fatty changes of the hepatocytes were also noted in adult $f_2$ females. Liver degeneration was noted in $f_1$ adults treated with 100 or 200 ppm vinylidene chloride and at all dose levels in $f_2$ adults. No dose-related malformations or changes in organ weights were revealed after necropsy of weanlings in any dose group or generation (Nitschke et al., 1980).

In summary, the three generation study by Nitschke et al. (1980) indicated that vinylidene chloride in drinking water of rats at 50, 100, or 200 ppm did not adversely affect reproduction or neonatal development. Neonatal survival was decreased in the $f_{3A}$ litters, but contradictory results were shown in the $f_{3B}$ and $f_{3C}$ litters. Histopathological examination revealed mild dose-related hepatotoxic effects in adult rats treated with vinylidene chloride, but no dose-related changes or malformations were seen in neonates. Reproductive capacities in adults did not appear altered by vinylidene choride exposure (Nitschke et al., 1980).

## 10.4 MUTAGENICITY

### 10.4.1 Mutagenicity in Bacteria

Numerous studies have shown vinylidene chloride to be a bacterial mutagen (Table 10-20). This is largely due to the use of vinylidene chloride as a positive control in studies analyzing the mutagenicity of chemicals that are gases at or near room temperature. A variety of exposure conditions have been utilized in these studies including standard plate incorporation, liquid suspension, and host mediation. The most commonly used method, however, involves exposure of bacteria to a defined atmosphere of vinylidene chloride in an enclosed vessel.

Bartsch et al. (1975) tested vinylidene chloride in the Ames assay using strains TA100 and TA1530, both of which respond to base-pair mutagens. Tester cells were exposed to 0, 0.2%, 2%, and 20% vinylidene chloride in air (v/v) for 4 hours. The concentrations of vinylidene chloride in the incubation medium after 2 hours of exposure to 0.2%, 2%, or 20% were $3.3 \times 10^{-4}$M, $3.3 \times 10^{-3}$M, or $3.3 \times 10^{-2}$M, respectively. These concentrations did not increase further with incubations of up to 7 hours. Dose-related increases in the mutant frequencies were seen at 0.2% and 2% vinylidene chloride and were dependent on the presence of phenobarbital-induced mouse liver S9 in the medium. The maximum response was seen in TA100 with an approximate 10-fold increase over baseline. The response decreased at 20% in both bacterial strains; the authors suggested that this may have been due to an inactivation of microsomal enzymes by vinylidene chloride. S9 derived from mouse liver was more effective at activation than S9 from kidney or lung. S9 derived from phenobarbital treated mice was more active than S9 derived from untreated animals, and S9 from mice was much more active than S9 from rats. The presence of N-acetyl-cysteine or N-acetyl-methionine in the medium decreased the mutagenic response by 80%. These latter

TABLE 10-20. SUMMARY OF MUTAGENICITY OF VINYLIDINE CHLORIDE (VDC): BACTERIA

| Reference | Test system | Chemical information | Dose | Results | Comments |
|---|---|---|---|---|---|
| Bartsch et al., 1975 | Ames assay: strains TA1530 and TA100 | Source: Merck-Schuchardt. Contained 0.3% methoxyphenol. | 0.2, 2, and 20% v/v in air for 4 h. | Positive in both strains. | S9 from mouse liver was more effective than kidney, which was mor effective than lung. Rat S9 was much less effective. Phenobarbital pretreatment increased the effect. |
| Bartsch et al., 1979 | Ames assay: strains TA1530 and TA100 | Source: Merck 99% pure. Contained 0.3% methoxyphenol. | 2% in air for 4 h. | Positive in both strains. | S9 from mouse liver. Also S9 from human liver of four donors. Human S9 only 1/5 as active. |
| Oesch et al., 1983 | Ames assay: strains TA1535, TA1537, TA98, TA100, and TA92. Reverse mutation in *E. coli* WP2 *uvrA*. | Source: BSAF, 99.996% pure. | 375, 2250, 4500, 10500, and 22500 ppm in air for 4 h. | Positive in all strains. | Efficiency of S9: mouse liver and hamster liver > rat liver > hamster kidney > male mouse kidney > rat kidney and female mouse kidney. |
| Jones and Hathway, 1978 | Ames assay: strain TA1535 | Source: Imperial Chemical Indust. LTD. | 5% in air for 72 h. | Positive | Efficiency of S9: mouse liver and kidney, induced by phenobarbital > induced rat liver and kidney > uninduced rat liver and kidney. |

(continued on the following page)

TABLE 10-20. (continued)

| Reference | Test system | Chemical information | Dose | Results | Comments |
|---|---|---|---|---|---|
| Greim et al., 1975 | Forward and reverse mutation in *E. coli* K12. Forward mutation to 5-methyltryptophan resistance and reverse mutation at the gal$^+$, arg$^+$, and nad$^+$ loci. | Source: Merck and Co. | 2.5 mM in medium for 2 h. | Negative at all loci in absence of S9. In the presence of S9, positive for gal, negative at other loci. | Dose used resulted in 75% cell survival. |
| Cerna and Kypenova, 1977 | Ames assay: strains TA1950, TA1951, TA1952, TA1535, TA1538, TA100, TA98. Female ICR mice as host mediators. | ? | Spot test: 1, 10, and 100%. | Spot test: positive at 100%, negative at lower doses. | Abstract only. |
| | | | Host mediation: 1/2 $LD_{50}$ and $LD_{50}$. | Host mediation: positive but not dose related. | |
| Baden et al., 1976 | Ames assay: strains TA98 and TA100 | Source: Dupont | 3% - 8 h in desiccator<br>3% - 1 h in medium<br>10% - 1 h in medium. | Negative minus S9 in TA100. Positive minus S9 in TA98. Positive in both strains with rat and human S9. | VDC used as positive control. |

(continued on the following page)

TABLE 10-20. (continued)

| Reference | Test system | Chemical information | Dose | Results | Comments |
|---|---|---|---|---|---|
| Baden et al., 1977 | Ames assay: strains TA100 and TA100 | ? | 3% in liquid incubation for 2 h. | Positive in both strains with Aroclor-induced rat S9. | Increase 4-5 x baseline. VDC used as positive control. |
| Baden et al., 1978 | Ames assay: strains TA100 and TA1535 | Source: Dupont | 3% in liquid incubation for 2 h. | Negative in both strains in absence of S9. Positive in both strains with Aroclor-induced rat S9. Positive in TA 100 with human S9. | VDC used as positive control. |
| Simmon et al., 1977 | Ames assay: strain TA100 | ? | 5% in air for 2, 3, 6, 9, and 12 h. | Positive, time related increases. | Little activity in absence of S9 mix. |
| Malaveille et al., 1977 | Ames assay: strain TA100 | Source: Merck-Schuchardt, 99% pure. Contained 0.3% methoxyphenol. | 2% in air for 2, 4, and 6 h. | Positive, time related increases. | Increase up to 10x background. S9 from phenobarbital-treated mice was more active than non-pretreated. |
| Waskell, 1978 | Ames assay: strain TA100 | Source: Dow Chemical Co. | 5% in air for 4 and 6 h. | Positive, time related increase in the presence of rat S9. | VDC used as positive control. |

compounds can act as free radical scavengers via their nucleophilic sulphur groups.

In a later publication, Bartsch et al. (1979) examined the effects of various chemical pretreatments on the activity of rat liver derived S9 mix. Pretreatment with phenobarbital and 3-methylcholanthrene resulted in increased activity (200% and 150%, respectively) while pretreatment with pregnenolone-16-alpha-carbonitrile, aminoacetonitrile, dibenamine, and dilsulfiram resulted in decreased activity. In this same study S9 derived from four human liver samples was also shown to be capable of activating vinylidene chloride to mutagenic products. On the whole, human liver S9 was only one-fifth as active as mouse S9.

In a recent study Oesch et al. (1983) compared the relative activities of S9 derived from various species and organs at activating vinylidene chloride to mutagenic products. The target organisms were five Ames tester strains (TA1535, TA1537, TA98, TA100, and TA92) and _E. coli_ WP2 _uvrA_ cells. Cultures were incubated at 375, 2250, 4500, 10500, and 22500 ppm for 6 hours. Positive results were seen at all doses with mouse liver S9 mix. The following sequence of activating potencies were observed in TA100: mouse liver and Chinese hamster liver > rat liver > human liver > Chinese hamster kidney > male mouse kidney > rat kidney and female mouse kidney. The last two sources generated S9s which were almost without activity. The authors demonstrated a correlation between vinylidene chloride treatment and the activity of the microsomal enzyme glutathione transferase. They suggested that this may be a contributing factor in species and sex differences noted for vinylidene chloride toxicity and carcinogenicity.

Another study that examined the effects of species, organ, and induction state on the activity of S9 mix was reported by Jones and Hathway (1978c). Ames strain TA1535 was exposed to 5% vinylidene chloride for 72 hours. S9 mixes derived from mouse liver and kidney were more efficient at activation than were S9 mixes derived from rat liver and kidney. In general, pretreatment of both rats and mice with Aroclor 1254 increased the activity of S9 mixes. S9 derived from uninduced marmoset liver failed to generate mutagenic products as did liver S9 from an uninduced human being. A second human-derived liver S9 mix from an individual after long-term phenobarbital treatment was active at generating mutagenic metabolites.

Baden and coworkers (Baden et al., 1976, 1977, 1978) have used both liquid incubation and gaseous exposure of several Ames strains to analyze the mutagenic properties of a variety of volatile anesthetics. In all these studies vinylidene chloride was used as a positive control. For the liquid suspension assay the concentration typically used was 3% for 1 hour, and the target organisms were TA100 and TA1535. For gaseous exposure TA98 and TA100 were incubated in an atmosphere of 3% vinylidene chloride for 8 hours. Typical responses in the presence of Aroclor-induced rat S9 mix averaged four to five times background. These authors have also shown that pretreatment of rats with an enzyme inducer such as Aroclor, greatly enhances the activity of liver-derived S9 mixes, and that S9 mix derived from human liver is also active at transforming vinylidene chloride to mutagenic products (Baden et al., 1976, 1978).

In addition to the studies cited above, a number of additional studies indicate that vinylidene chloride is positive in the Ames assay as well as in other bacterial tests. Simmon et al. (1977) showed a time related increase in the number of TA100 revertants exposed to an atmosphere of 5% vinylidene chloride in air for 3, 6, 9, and 12 hours. Little activity was observed in the absence of S9 mix (Figure 10-6).

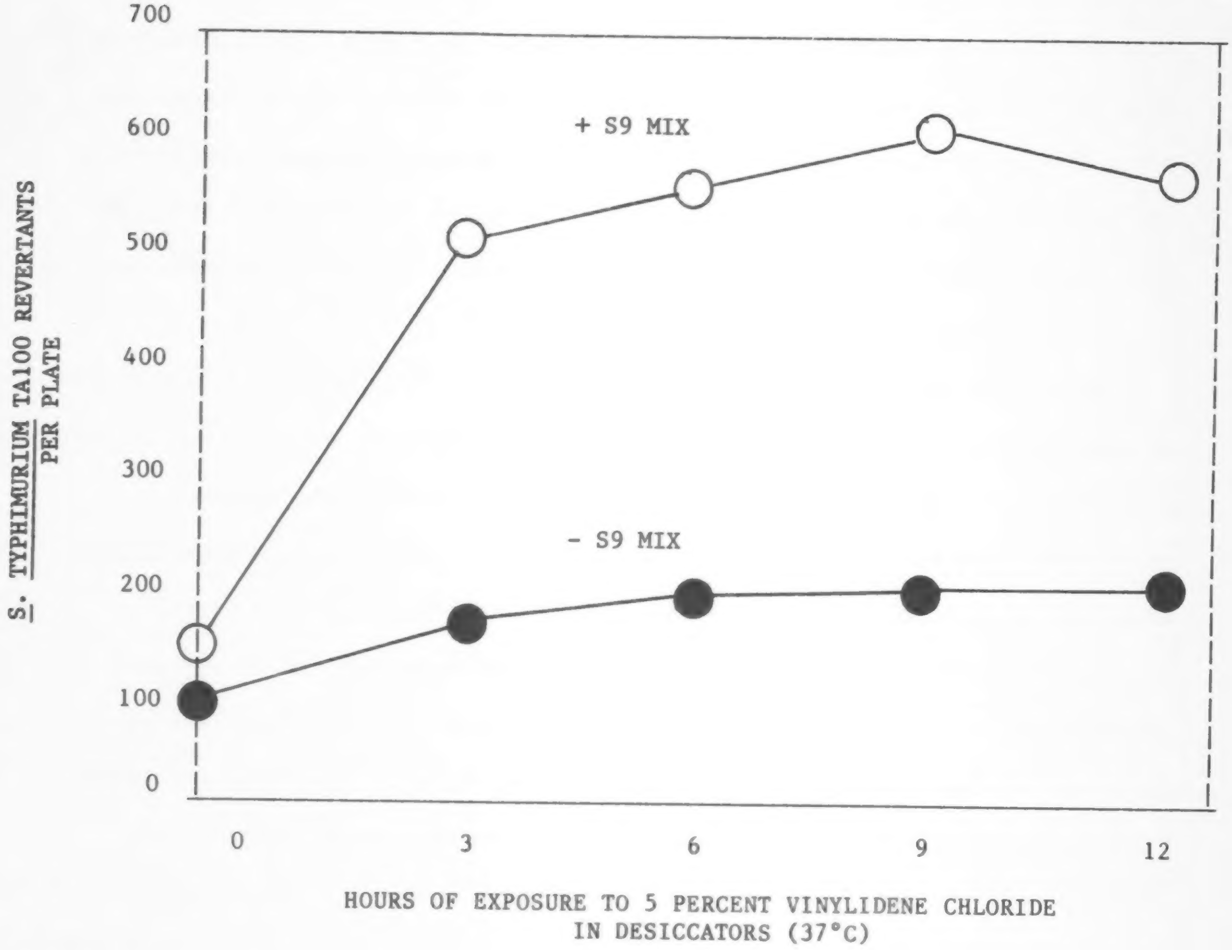


Figure 10-6. Mutagenic activity of vinylidene chloride in the presence and in the absence of metabolic activation.

SOURCE: Simmon et al., 1977.

In a study characterizing the stability of metabolic activation systems, Malaveille et al. (1977) showed time dependent increases in the numbers of TA100 revertants after incubation in an atmosphere of 2% vinylidene chloride in air for 2, 4, and 6 hours. The largest increases in the number of revertants were up to ten times background. In agreement with other studies, S9 derived from phenobarbital-treated mice was more active than S9 from untreated animals.

Similarly, Waskell (1978) showed a time dependent increase in the numbers of TA100 revertants after incubation in an atmosphere of 5% vinylidene chloride in air for 4 and 6 hours. This activity was dependent on the presence of a rat liver S9 mix.

Greim et al. (1975), using a liquid suspension protocol, examined vinylidene chloride-induced reverse mutation at three loci (gal, arg, nad) and forward mutation at one locus (MTR) in E. coli K12. Cells were incubated in medium containing 2.5 mM vinylidene chloride for 2 hours and tested for reversion to prototrophy (gal, arg, nad) or resistance to 5-methyl-tryptophan (MTR). A significant increase in the mutant frequency was seen only at the arg locus (2.3-times control) when cells were incubated in the presence of phenobarbital-pretreated mouse S9 mix.

In a study reported in abstract form only, Cerna and Kypenova (1977) reported that vinylidene chloride was positive in the bacterial spot test and the Ames assay using host-mediated activation. In the spot test, 0.05 mL of vinylidene chloride at 100%, 10%, and 1% was tested using strains TA1950, TA1951, TA1952, TA1535, TA1538, TA100, and TA98. Positive results in strains sensitive to both base-pair and frameshift mutagens were seen only for 100% vinylidene chloride. In the host-mediated assay, female ICR mice were injected with vinylidene chloride at the $LD_{50}$ and one-half of the $LD_{50}$, and mutagenicity was tested in strains TA1950, TA1951, and TA1952. Although positive results were noted, they were inversely related to dose.

There has been speculation as to the nature of the ultimate mutagen formed during metabolic activation of vinylidene chloride. Bartsch et al. (1975) have suggested that an alkylating intermediate is formed, since mutations occur in *S. typhimurium* strain TA1530 which is reported by Ames et al. (1973) to be specifically reverted by monofunctional alkylating agents. Nevertheless, an alkylating compound was not identified in the trapping agent, 4-(4'-nitrobenzyl)-pyridine, when a gas that contained vinylidene chloride was passed through a mouse S9 activating system, even though this system provided activated metabolites that mutated *S. typhimurium* strain TA100 (Barbin et al., 1978). It has also been suggested that an unstable oxirane, possibly 1,1-dichloroethylene oxide, may be formed during metabolism and that this metabolite plays a significant role in the mutagenic activity of vinylidene chloride (Greim et al., 1975; Bonse et al., 1975; Bartsch et al., 1979). Presently, however, there is little evidence identifying the exact metabolic intermediates that constitute the ultimate mutagenic form(s) of vinylidene chloride.

### 10.4.2 Mutagenicity in Yeast and Plants

Vinylidene chloride was tested for the induction of point mutations and mitotic gene conversion in the diploid strain (D7) of the yeast *Saccharomyces cerevisiae* (Bronzetti et al., 1981, Table 10-21). Cells were treated in a suspension containing 10, 20, 30, 40, or 50 mM vinylidene chloride in the presence and absence of mouse liver S10 mix. In a second procedure employing host activation, yeast cells were injected in the retroorbital sinus of male Swiss albino mice (CD strain) and collected after 4 hours from the liver, lungs, and kidneys.

In the suspension assay a dose-related increase in revertants (Ilv+) and

TABLE 10-21. SUMMARY OF MUTAGENICITY OF VINYLIDINE CHLORIDE (VDC): YEAST AND PLANTS

| Reference | Test system | Chemical information | Dose | Results | Comments |
|---|---|---|---|---|---|
| Bronzetti et al., 1981 | Reverse mutation and gene conversion in Saccharomyces. Activation either with mouse S10 mix or host mediated in male Swiss albino CD mice. | Source: Fluka, 99.75% pure. Containing 0.02% 4-methoxyphenol. | 10, 20, 30, 40, 50 mM for 2 h in suspension or for host mediation. 400 mg/kg acute or 23 x 100 mg/kg given 5 days/week plus 200 mg/kg on day of assay. Total dose 2500 mg/kg. Cells collected after 4 h exposure. | Suspension: Dose related increase in revertants and convertants in the presence of Aroclor 1254 pretreated mouse S10.<br>Host mediation: In acute study, positive for revertants and convertants for cells isolated from liver and kidney and negative for cells from lung.<br>Similar results in chronic study.<br>4-methoxyphenol was negative at a concentration 10x greater than found in VDC. | Increases were seen when cell survival was above 50%.<br>Acute study: liver-10x increase in convert 12x increase in revert.<br>Kidney - 6x increase in convert, 5x increase in revert.<br>Chronic study: liver - 13x increase in convert and 2x increase in revert. |
| Van't Hof and Schairer, 1982 | Forward mutation: score change in flower color from blue to pink. | ? | 1288 ppm for 6 h.<br>22 ppm for 24 h. | Negative for 1288 ppm.<br>Positive for 22 ppm. | These results seem anomalous but cannot be explained since the results come from a Gene-Tox publication and the original source is not cited. |

convertants ($Trp^+$) was seen in the presence of S10 isolated from Aroclor 1254 treated mice. The increases in revertants (up to eight times control) and convertants (up to five times control) were noted at vinylidene chloride concentrations that permitted more than 50% cell survival.

In the host mediated assay, mice were given an acute oral dose of 400 mg/kg vinylidene chloride, or were chronically treated (100 mg/kg, 5 days/week with a total of 23 administrations, plus 200 mg/kg on the day of the assay; total dose was 2500 mg/kg). In the acute protocol there was a 10-fold increase in the number of convertants and a 12-fold increase in the number of revertants isolated from the liver, a 6-fold increase in convertants and a 5-fold increase in revertants isolated from the kidney, while neither end point was increased in cells isolated from the lung. In chronically exposed mice, convertants were increased 13-fold and 6-fold in the liver and kidney, respectively, and did not increase in the lung, while revertants increased 6-fold and 30-fold in the liver and kidney, respectively, and again were negative in the lung. 4-methoxyphenol, which is often added to vinylidene chloride as an antioxidant, was negative at a concentration 10-fold higher than normally found in vinylidene chloride.

The results of testing vinylidene chloride in tradescantia were reported in a Gene-Tox publication that evaluated the data base associated with testing in this plant (Van't Hof and Schairer, 1982, Table 10-21). The hybrid clone of tradescantia used in these studies (4430) is heterozygous at a flower-color locus. The dominant gene results in a blue flower, while inactivation of this locus allows the expression of the recessive allele resulting in a pink flower. Vinylidene chloride was reported to give negative results after exposure to 1288 ppm for 6 hours, while positive results ($p < 0.05$) were reported after exposure to 22 ppm for 24 hours. Clearly, these results are difficult to reconcile. Unfortunately, the original reference from which these results were derived was not cited in the Gene-Tox report.

### 10.4.3 Mutagenicity in Cultured Mammalian Cells

Vinylidene chloride was tested for its ability to induce gene mutations at the hypoxanthine guanine phosphoribosyl transferase and sodium-potassium ATPase loci in V79 cells (Drevon and Kuroki, 1979; Table 10-22). Cultured cells were exposed to 2% and 10% vinylidene chloride in air for 5 hours. No cytotoxicity and no increase in the mutant frequencies were noted for vinylidene chloride-exposed cells incubated in the presence of S15 from mouse liver or in the absence of microsomal enzymes. Cells exposed to vinylidene chloride in the presence of rat S15 showed 76% and 96% cytotoxicity at concentrations of 2% and 10%, respectively. Again, there were no increases in the frequencies of mutants. Both rats and mice had been pretreated with phenobarbital, and positive results were reported when vinyl chloride was similarly tested.

### 10.4.4 Mutagenicity in Mammalian Cells *In Vivo*

The only report on the cytogenetic effects of vinylidene chloride was published in abstract form (Cerna and Kypenova, 1977, Table 10-22). Female ICR mice were given vinylidene chloride in a single dose (one-half of the $LD_{50}$) or in repeated applications (one-sixth of the $LD_{50}$, five intraperitoneal doses given at one day intervals). Animals were killed 6, 24, and 48 hours following the last injection, and the bone marrow was analyzed for aberrations. No increase in chromosomal aberrations was found in any treatment group.

The capacity of vinylidene chloride to alkylate DNA and induce unscheduled DNA synthesis *in vivo* was studied by Reitz et al. (1980, Table 10-22). These investigators exposed CD-1 mice and Sprague-Dawley rats to $^{14}C$-labeled vinylidene chloride vapor for 6 hours at 10 and 50 ppm (mouse only). DNA was then extracted

TABLE 10-22. SUMMARY OF MUTAGENICITY OF VINYLIDENE CHLORIDE (VDC): MAMMALIAN CELLS

| Reference | Test system | Chemical information | Dose | Results | Comments |
|---|---|---|---|---|---|
| Drevon and Kuroki, 1979 | Forward gene mutation at the HGPRT locus and membrane sodium-potassium ATPase locus in V79 cells. | Source: Merck-Schuchardt. Contained 0.3% methoxyphenol. | 2% or 10% in air for 5 h. | Negative for mutations at both loci both in the presence and absence of mouse and rat liver S15 mix. | Mouse and rats pre-treated with phenobarbital. S15 from rat caused 76% toxicity at 2% and 96% toxicity at 10%. Vinyl chloride was positive in this study. |
| Cerna and Kypenova, 1977 | Cytogenetic analysis in female ICR mouse bone marrow. | ? | Acute: 1/2 $LD_{50}$.<br>Multiple: 1/6 $LD_{50}$ x 5, 1 h/day, 5 days. | Negative | Abstract only. |
| Reitz et al., 1980 | Binding of radiolabeled VDC to DNA of liver and kidney in CD-1 mice and Sprague-Dawley rats. | Source: Saran and Converted Products Res., 99.95% pure. | 50 ppm and 10 ppm in air for 6 h. | Binding: higher levels seen in kidney than liver, more binding in mice than rats. | There were serious problems in the procotol for *in vivo* UDS, details are given in text. |
|  | UDS *in vivo* in liver and *kidney* of CD-1 mice. |  |  | UDS: significant increase in kidneys of mice given 50 ppm. | DMN was positive control. |

(continued on the following page)

TABLE 10-22. (continued)

| Reference | Test system | Chemical information | Dose | Results | Comments |
|---|---|---|---|---|---|
| Anderson et al., 1977 | Dominant lethality in male CD mice. | Source: ICI LTD. | 10, 30, and 50 ppm, 6 h/day, 5 days. | Negative for: number of implants, number of females with ≥ 1 early deaths/pregnancy, early deaths as a % of total implants. | Vinyl chloride was also negative. There was reduced fertility at 50 ppm. The positive controls, cyclophosphamide and EMS gave positive responses. |
| Short et al., 1977 | Dominant lethality in male CD rats. | ? | 55 ppm, 6 h/day, 5 days/week, 11 weeks. | Negative for pre- and postimplantation loss. | Decrease in fertility was noted. |

from the kidneys and livers and analyzed for covalently bound radiolabel. The greatest level of binding was found in the kidneys of mice exposed to 50 ppm (30 alkylations/$10^6$ nucleotides) followed by the kidneys of mice exposed to 10 ppm (11 alkylations/$10^6$ nucleotides). Livers of exposed mice showed lower but still detectable levels of binding. Rats exposed to 10 ppm vinylidene chloride showed 2.0 and 0.87 alkylations/$10^6$ nucleotides for the kidney and liver, respectively. Radioactivity was eliminated from the kidney and liver of 50 ppm mice in a biphasic manner with a half-life of 83 and 91 hours, respectively. Rates of elimination were dose related--the lower doses being eliminated more rapidly.

Unscheduled DNA systhesis (UDS) was measured in the kidneys and livers of mice exposed for 6 hours to 10 and 50 ppm vinylidene chloride. Immediately after vinylidene chloride exposure, the animals were injected with $^3$H-thymidine alone or with $^3$H-thymidine plus hydroxyurea. This latter drug is given "to block most of the replicative DNA synthesis." The authors claimed a significant increase in UDS only in kidneys of mice exposed to 50 ppm. No effects were claimed for liver at 50 ppm or for either organ at 10 ppm. The validity of all these data is questionable for two reasons. First, the control values for normal DNA synthesis varied from 12.9 to 80.5 cpm/mg DNA in the liver of otherwise untreated mice and from 2.57 to 18.4 cpm/mg DNA in the livers of hydroxyurea-treated mice. Second, the effects of hydroxyurea on normal DNA synthesis and UDS could not be separated since this compound failed to suppress all scheduled DNA synthesis. Thus, in the one instance where the authors claimed a significant increase in UDS, the specific activity of DNA was lower than in the control.

The effect of vinylidene chloride on the induction of dominant lethals has been studied in both mice (Anderson et al., 1977) and rats (Short et al., 1977, Table 10-22). In the mouse study, 8- to 12-week-old CD males were exposed to 10, 30, and 50 ppm in air 6 hours/day for a total of 5 days. Matings began immediately after dosing and continued (one male with two virgin females) for a total of 8 weeks. Females were killed in midgestation after 14 to 16 days of pregnancy. Vinylidene chloride at 50 ppm reduced fertility in treated males up to 6 weeks following treatment. In none of the treatment groups were there significant effects on the number of implants, the number of females with $\geq$ one early death, the mean number of early deaths per pregnancy, or the early deaths as a percent of total implants. Vinyl chloride, which was also tested in this study, was also reported to be negative. The positive controls, cyclophosphamide, which was given intraperitoneally and ethylmethanesulphonate, which was given per os, gave positive responses.

In the rat study, male CD rats were exposed to 55 ppm vinylidene chloride in air 6 hours/day, 5 days/week, for 11 weeks (Short et al., 1977). During week 11 of exposure, each male was housed with two unexposed virgin females for at least seven successive evenings or until the male mated with two females. Females were killed on day 13 of gestation, and the number of corpora lutea and implants were determined. As in the mouse study there was a significant decline in the number of pregnant females produced by exposed males. No effect was observed regarding the number of corpora lutea per dam, number of implants per dam, percent implants per corpora lutea, or percent viable embryos per total implants.

### 10.4.5 Summary

A large number of studies indicate the vinylidene chloride is mutagenic to bacteria and that this activity is largely dependent on microsomal activation. Vinylidene chloride was reported to produce positive results for gene reversion and conversion in yeast, which was also dependent on metabolic activation, and was positive in tradescantia. In mammalian systems vinylidene chloride failed to induce gene mutations in V79 cells at two seperate loci, failed to induce chromosomal aberrations in mouse bone marrow *in vivo*, and failed to induce dominant lethals in either mice or rats. Vinylidene chloride was found to alkylate the DNA of mice exposed through inhalation and may have caused unscheduled DNA synthesis in the kidneys of similarly exposed mice.

Analysis of the data relating to the potential of vinylidene chloride to behave as a human germ-cell mutagen indicates that based on the criteria established in the Agency's Proposed Guidelines for Mutagenicity Risk Assessment, the evidence at the present time is classified as limited. This designation indicates that there are insufficient data on either mutagenicity or interaction with germ cells to classify the evidence as either sufficient or suggestive of potential germ-cell mutagenicity. However, available data also do not permit the classification of vinylidene chloride as a non-germ cell mutagen.

## 10.5 CARCINOGENICITY

### 10.5.1 Animal Studies

There have been a number of laboratory investigations of the carcinogenic potential of vinylidene chloride. These studies have been performed using rats, mice, and hamsters, with vinylidene chloride administered by inhalation, gavage, incorporation into drinking water, subcutaneous injection, and topical applications. These results are summarized in Table 10-23, and have also been summarized by Chu and Milman (1981). Of the studies performed, only the results reported by Maltoni et al. (1985) indicated a statistically significant positive carcinogenic effect resulting from vinylidene chloride treatment.

Maltoni et al. (1985) exposed Sprague-Dawley rats, Swiss mice, and Chinese hamsters to vinylidene chloride (99.95% pure, Table 10-24) in an inhalation chamber for 4 hours daily, 4 to 5 days a week for 12 months. The exposure concentration used depended on the species' susceptibility to vinylidene chloride's toxic action; the highest tolerated dose in mice and rats was used as the maximum concentration. The exposure level for hamsters was not selected on the basis of achieving a maximum tolerated dose. Rats, initially 16 weeks old, were exposed to vinylidene chloride at 150, 100, 50, 25, and 10 ppm (Table 10-25), while mice, initially 16 weeks old or 9 weeks old, were chronically exposed to vinylidene chloride at 25 and 10 ppm (Table 10-26), and hamsters, initially 28 weeks old, were chronically exposed to a concentration of vinylidene chloride at 25 ppm (Table 10-27). In addition, rats, initially 9 weeks old, were treated by gavage with 20, 10, 5, and 0.5 mg/kg/day of vinylidene chloride dissolved in olive oil (Table 10-28). Body weights were recorded every 2 weeks during the 12-month treatment period and monthly thereafter. The studies were completed in 137 weeks for the inhalation study in rats, 147 weeks for the ingestion study in rats, 121 weeks for the inhalation study in

TABLE 10-23. RESULTS OF CARCINOGENICITY BIOASSAYS OF VINYLIDENE CHLORIDE

| Species | Dose | Route of administration | Total duration of observation | Findings | Reference |
|---|---|---|---|---|---|
| Sprague-Dawley rats | 10, 25, 50, 100, and 150 ppm 4 to 5 days/week for 12 months | Inhalation, 4 h/day | 137 weeks | Statistically significant increase in total mammary tumors, but not carcinomas alone, only at 10 and 100 ppm, no dose response | Maltoni et al., 1985 |
| Swiss mice | 10 and 25 ppm 4 to 5 days/week for 12 months | Inhalation, 4 h/day | 121 weeks | Kidney carcinomas at 25 ppm in males (none in controls)<br>Statistically significant increase in mammary carcinomas in females, no dose response | Maltoni et al., 1985 |
| Chinese hamsters | 25 ppm 4 to 5 days/week for 12 months | Inhalation, 4 h/day | 157 weeks | No statistically significant increase | Maltoni et al., 1985 |
| Sprague-Dawley rats | 20, 10, 5, and 0.5 mg/kg for 12 months | Gavage, daily | 147 weeks | No statistically significant increase | Maltoni et al., 1985 |
| Wistar rats | 200 ppm for 6 months, followed by 100 ppm for 6 months, 5 days/week | Inhalation, 4 h/day | Lifetime | No statistically significant increase | Viola and Caputo, 1977 |
| Sprague-Dawley rats | 100 and 75 ppm 5 days/week for 12 months | Inhalation, 4 h/day | Lifetime | No statistically significant increase | Viola and Caputo, 1977 |
| CD-1 mice | 55 ppm 5 days/week for 12 months | Inhalation, 6 h/day | 12 months | No statistically significant increase | Lee et al., 1978 |
| CD rats | 55 ppm 5 days/week for 12 months | Inhalation, 6 h/day | 12 months | No statistically significant increase | Lee et al., 1978 |
| Sprague-Dawley rats | 25 and 75 ppm for 24 months | Inhalation | 104 weeks | No statistically significant increase | McKenna et al., 1982 |

(continued on the following page)

TABLE 10-23. (continued)

| Species | Dose | Route of administration | Total duration of observation | Findings | Reference |
|---|---|---|---|---|---|
| Sprague-Dawley rats | 50, 100, and 200 ppm in drinking water | Ingestion | 104 weeks | No statistically significant increase | Quast et al., 1983 |
| Fischer 344 rats | 5 mL/kg of a 1000 or 200 ppm solution | Gavage, 5 days/week | 103 weeks | No statistically significant increase | NCI/NTP, 1982 |
| B6C3F1 mice | 10 mL/kg of a 1000 or 200 ppm solution | Gavage, 5 days/week | 103 weeks | No statistically significant increase | NCI/NTP, 1982 |
| CD mice | 55 ppm, 5 days/ week 1, 3, or 6 months | Inhalation, 6 h/day | 13, 15, or 18 months | No statistically significant increase | Hong et al., 1981 |
| CD rats | 55 ppm, 5 days/ week 1, 3, 6, or 10 months | Inhalation, 6 h/day | 13, 15, 18, or 22 months | No statistically significant increase | Hong et al., 1981 |
| Ha:ICR Swiss | 121 mg/mouse | Skin application, 3 times/week | Lifetime | No tumors | Van Duuren et al., 1979 |
| Ha:ICR Swiss | 2 mg/mouse | Subcutaneous injection, once/week | Lifetime | No tumors at site of injection or in other organs | Van Duuren et al., 1979 |
| Sprague-Dawley rats | 10, 25, 50, 100, and 150 ppm | Inhalation, 4 to 5 days/week | 52 weeks | No brain tumors | Maltoni et al., 1982 |
| Sprague-Dawley rats | 0.5, 5, 10, and 20 mg/kg/day | Gavage, 5 days/week | 52-59 weeks | No brain tumors | Maltoni et al., 1982 |

## TABLE 10-24. GAS CHROMATOGRAPHY ANALYSIS OF VINYLIDENE CHLORIDE

| Compound | Amount |
|---|---|
| Vinylidene chloride (1,1-Dichloroethylene) | 999.5 g/kg |
| 1,2-Dichloroethylene trans | 0.4 g/kg |
| Acetone | 0.1 g/kg |
| Methylene chloride (Dichloromethane) | 0.05 g/kg |
| Monochloroacetylene and Dichloroacetylene | 0.02 g/kg |
| Paramethoxyphenol (as stabilizer) | 200 ppm |

SOURCE: Maltoni et al., 1985.

## TABLE 10-25. EXPERIMENT DT401: EXPOSURE BY INHALATION TO VINYLIDENE CHLORIDE IN AIR AT 150, 100, 50, 25, AND 10 PPM, 4 HOURS DAILY, 4-5 DAYS WEEKLY, FOR 52 WEEKS

| Group nos. | Concentration | Animals (Sprague-Dawley rats, 16 weeks old at start) | | |
|---|---|---|---|---|
| | | Males | Females | Total |
| I | 200 - 150 ppm[a] | 60 | 60 | 120 |
| II | 100 ppm | 30 | 30 | 60 |
| III | 50 ppm | 30 | 30 | 60 |
| IV | 25 ppm | 30 | 30 | 60 |
| V | 10 ppm | 30 | 30 | 60 |
| VI | No treatment | 100 | 100 | 200 |
| Total | | 250 | 280 | 560 |

[a] Two treatments only because of the high toxicity of this dose level.

SOURCE: Maltoni et al., 1985.

TABLE 10-26. EXPOSURE BY INHALATION TO VINYLIDENE CHLORIDE IN AIR AT 200, 100, 50, 25, AND 10 PPM, 4 HOURS DAILY, 4-5 DAYS WEEKLY, FOR 52 WEEKS

| Group nos. | Treatment | | Animals<br>Swiss mice 16 weeks old (groups I, II, III, IV, V, VI) and 9 weeks old (groups IV bis and VII) | | |
|---|---|---|---|---|---|
| | Concentration | Length | Males | Females | Total |
| I | 200 ppm | 2 days[d] | 60 | 60 | 120 |
| II | 100 ppm | 2 days[d] | 30 | 30 | 60 |
| III | 50 ppm | 1 week[d] | 30 | 30 | 60 |
| IV | 25 ppm | 52 weeks | 30 | 30 | 60 |
| IV bis[a] | 25 ppm | 52 weeks | 120 | 120 | 240 |
| V | 10 ppm | 52 weeks | 30 | 30 | 60 |
| VI | No treatment (Controls)[b] | | 100 | 100 | 200 |
| VII | No treatment (Controls)[c] | | 90 | 90 | 180 |
| Total | | | 490 | 490 | 980 |

[a] The treatment started two weeks later than in other groups.

[b] Controls to the groups I, II, III, IV, V.

[c] Controls to the group IV bis.

[d] The treatment was interrupted because of the high toxic effects and high mortality.

SOURCE: Maltoni et al., 1985.

TABLE 10-27. EXPERIMENT BT405: EXPOSURE BY INHALATION TO VINYLIDENE CHLORIDE IN AIR AT 25 PPM, 4 HOURS DAILY, 4-5 DAYS WEEKLY, FOR 52 WEEKS

| Group nos. | Concentration | Animals (Chinese hamsters, 28 weeks old at start) | | |
|---|---|---|---|---|
| | | Males | Females | Total |
| I | 25 ppm | 30 | 30 | 60 |
| II | No treatment (Controls) | 18 | 17 | 35 |
| Total | | 48 | 47 | 95 |

SOURCE: Maltoni et al., 1985.

TABLE 10-28. EXPERIMENT BT403: EXPOSURE BY INGESTION (STOMACH TUBE) TO VINYLIDENE CHLORIDE IN OLIVE OIL AT 20, 10, AND 5 MG/KG BODY WEIGHT, ONCE DAILY, 4-5 DAYS WEEKLY, FOR 52 WEEKS

| Group nos. | Concentration | Animals (Sprague-Dawley rats, 9 weeks old at start) | | |
|---|---|---|---|---|
| | | Males | Females | Total |
| I | 20 mg/kg | 50 | 50 | 100 |
| II | 10 mg/kg | 50 | 50 | 100 |
| III | 5 mg/kg | 50 | 50 | 100 |
| IV | None (olive oil alone) (Controls) | 100 | 100 | 200 |
| Total | | 250 | 250 | 500 |

EXPERIMENT BT404: EXPOSURE BY INGESTION (STOMACH TUBE) TO VINYLIDENE CHLORIDE IN OLIVE OIL AT 0.5 MG/KG BODY WEIGHT, ONCE DAILY, 4-5 DAYS WEEKLY, FOR 52 WEEKS

| Group nos. | Concentration | Animals (Sprague-Dawley rats, 9 weeks old at start) | | |
|---|---|---|---|---|
| | | Males | Females | Total |
| I | 0.5 mg/kg | 50 | 50 | 100 |
| II | None (olive oil alone) (Controls) | 82 | 77 | 159 |
| Total | | 132 | 127 | 259 |

SOURCE: Maltoni et al., 1985.

mice, and 157 weeks for the inhalation study in hamsters. Animals were allowed to survive until spontaneous death. Each animal was necropsied, and normally appearing tissues and organs, as well as tumors and lesions, were examined histopathologically.

No treatment-related effect on body weights in rats was evident. Body weight data for mice and hamsters were not reported, but no effect attributable to treatment was noted.

There were no significant ($p < 0.05$) differences in tumor development between control and treated hamsters.

A number of neoplastic lesions were observed in control and treated rats, with no treatment- or dose-related effect in evidence from either inhalation exposure or gavage administration. The 1977 report by Maltoni et al. presented interim (82 weeks) results showing an increased incidence of mammary tumors in treated rats compared to controls in the inhalation study; however, at that time, a clear dose-related effect was not evident, spontaneous mammary tumor incidence in female controls was 32%, and histopathologic diagnosis of the mammary tumors was ongoing. The final mammary tumor data for female rats in this study, as described in the Maltoni et al. (1985) report and Table 10-29 herein, show statistically significant ($p < 0.05$) increases in the total number of tumor-bearing animals in the 10 ppm and 100 ppm groups compared to controls, and in the number of animals with fibromas and fibroadenomas in each treatment group compared to controls. However, there was no clear dose-related increase in mammary tumor incidence, latency time for mammary tumor formation was similar among all groups, there was a high (61%) incidence of spontaneously formed mammary tumors in controls, and mammary carcinoma incidence in treated groups was not significantly ($p < 0.05$) different from and was actually consistently less than that of controls. Hence, the evidence for a carcinogenic

TABLE 10-29. EXPERIMENT BT401: EXPOSURE BY INHALATION TO VINYLIDENE CHLORIDE IN AIR AT 150, 100, 50, 25, AND 10 PPM, 4 HOURS DAILY, 4-5 DAYS FOR 52 WEEKS. RESULTS AFTER 137 WEEKS (END OF EXPERIMENT).

DISTRIBUTION OF THE DIFFERENT TYPES OF MAMMARY TUMORS

| Group nos. | Concentrations | Animals (Sprague-Dawley rats, 16 weeks old at start) | | | Mammary Tumors[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Histologically Examined | | | | | | | |
| | | | | | | | | | | | Histotype | | | | | |
| | | | | | | | | | | | Fibromas and fibroadenomas | | | Carcinomas | | |
| | | Sex | No. at start | Corrected number[b] | Total No. | %[c] | Average latency time (weeks)[d] | No. of tumors/ tumor-bearing animals | Total No. | %[e] | No. | %[f] | Average latency time (weeks)[d] | No. | %[f] | Average latency time (weeks)[d] |
| I | 150 ppm | M | 60 | 60 | 8 | 13.3 | 97±14 | 1.0 | 8 | 100.0 | 6 | 75.0 | 109± 8 | 1 | 12.5 | 26 |
| | | F | 60 | 60 | 44 | 73.3 | 82± 3 | 1.5 | 43 | 97.7 | 38[g] | 88.4 | 83± 3 | 9 | 20.9 | 78± 8 |
| | | M and F | 120 | 120 | 52 | 43.3 | 82± 3 | 1.4 | 51 | 98.1 | 44 | 86.3 | 86± 3 | 10 | 19.6 | 73± 8 |
| II | 100 ppm | M | 30 | 30 | 5 | 16.7 | 104± 9 | 1.0 | 5 | 100.0 | 5 | 100.0 | 104± 9 | 0 | - | - |
| | | F | 30 | 30 | 25[g] | 83.3 | 82± 4 | 1.7 | 23 | 92.0 | 21[g] | 91.3 | 83± 5 | 3 | 13.0 | 102±10 |
| | | M and F | 60 | 60 | 30 | 50.0 | 85± 4 | 1.6 | 28 | 93.3 | 26 | 92.8 | 87± 4 | 3 | 10.7 | 102±10 |
| III | 50 ppm | M | 30 | 30 | 7 | 23.3 | 106± 5 | 1.0 | 7 | 100.0 | 7 | 100.0 | 106± 5 | 0 | - | - |
| | | F | 30 | 30 | 23 | 76.7 | 79± 4 | 1.9 | 22 | 95.6 | 21[g] | 95.4 | 82± 4 | 1 | 4.5 | 68 |
| | | M and F | 60 | 60 | 30 | 50.0 | 86± 4 | 1.7 | 29 | 96.7 | 28 | 96.5 | 88± 4 | 1 | 3.4 | 68 |
| IV | 25 ppm | M | 30 | 28 | 4 | 14.3 | 103±10 | 1.0 | 4 | 100.0 | 4 | 100.0 | 103±10 | 0 | - | - |
| | | F | 30 | 30 | 21 | 70.0 | 86± 4 | 1.6 | 20 | 95.2 | 20[g] | 100.0 | 87± 4 | 4 | 20.0 | 82±10 |
| | | M and F | 60 | 58 | 25 | 43.1 | 88± 4 | 1.5 | 24 | 96.0 | 24 | 100.0 | 90± 4 | 4 | 16.7 | 82±10 |
| V | 10 ppm | M | 30 | 29 | 3 | 10.3 | 81±23 | 1.0 | 3 | 100.0 | 3 | 100.0 | 81±23 | 0 | - | - |
| | | F | 30 | 30 | 28[g] | 93.3 | 83± 4 | 1.6 | 24 | 85.7 | 24[g] | 100.0 | 85± 4 | 5 | 20.8 | 90±14 |
| | | M and F | 60 | 59 | 31 | 52.5 | 81± 4 | 1.5 | 27 | 87.1 | 27 | 100.0 | 85± 4 | 5 | 18.5 | 90±14 |
| VI | No treatment (Controls) | M | 100 | 87 | 11 | 12.6 | 115± 6 | 1.0 | 11 | 100.0 | 11 | 100.0 | 115± 6 | 0 | - | - |
| | | F | 100 | 99 | 61 | 61.6 | 87± 2 | 1.5 | 56 | 91.8 | 44 | 78.6 | 88± 3 | 16 | 28.6 | 95± 5 |
| | | M and F | 200 | 186 | 72 | 38.7 | 91± 3 | 1.4 | 67 | 93.0 | 55 | 82.1 | 93± 3 | 16 | 23.9 | 95± 5 |
| Total | | | 560 | 543 | | | | | | | | | | | | |

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[a] Two or more tumors of the same and/or different types (fibroadenomas, carcinomas, sarcomas, carcinosarcomas) may be present in the same animals. A carcinosarcoma was found in one male in the 150 ppm group, and no animals were observed to have sarcomas.

[b] Alive animals after 10 weeks, when the first tumor (a leukemia) was observed.

[c] The percentages refer to the corrected numbers.

[d] Average age at the onset of the first mammary tumor per animal, detected at the periodic control or at autopsy.

[e] The percentages refer to total numbers of animals bearing mammary tumors.

[f] The percentages refer to total numbers of animals bearing mammary tumors, histologically examined.

[g] Statistically significant increase compared to control by chi-square test ($p < 0.05$). Comparisons are made between numbers with tumors/corrected numbers.

SOURCE: Maltoni et al., 1985.

effect of inhaled vinylidene chloride in female Sprague-Dawley rats, as mammary tumors, in this study would appear to be inconclusive.

In male Swiss mice, kidney adenocarcinomas were observed following inhalation exposure to vinylidene chloride at 25 ppm (Table 10-30). In female Swiss mice, mammary carcinoma incidence in both the 10 ppm and combined 25 ppm groups compared to combined controls was significantly ($p < 0.01$) increased, and a small incidence of mammary carcinomas was evident in females exposed to vinylidene chloride at 50, 100, or 200 ppm for 1 week or less (Table 10-30). The incidence of pulmonary adenomas was significantly ($p < 0.01$) increased in male and female mice exposed to vinylidene chloride at 10 and 25 ppm compared to controls (Table 10-30); however, pulmonary carcinomas were not found in any of the mice.

Maltoni et al. (1985) concluded that the kidney tumors in male mice developed in response to vinylidene chloride treatment, particularly since no corresponding spontaneous tumors were noted in the 190 control male mice. Maltoni et al. (1985) also concluded that the toxic and carcinogenic effects of vinylidene chloride may depend on species, strain, and sex of the tested animals, and that the observed kidney tumors in Swiss mice may be a strain-specific phenomenon. It has been noted that male mice of several strains are particularly sensitive to the toxic effects of vinylidene chloride (Maltoni et al., 1977; Maltoni, 1977; Maltoni and Patella, 1983; Maltoni et al., 1985; Henck et al., 1979, 1980). The kidney and liver appear to be the major target organs for vinylidene chloride toxicity. Many authors have postulated that a reactive metabolite is responsible for both effects of vinylidene chloride. The primary target organ for vinylidene chloride-induced toxicity in the male mouse is the kidney (McKenna et al., 1977, 1978a, b), although the liver is also a target organ for toxicity in mice. The kidney is also the target organ

TABLE 10-30. EXPERIMENT BT402: EXPOSURE BY INHALATION TO VINYLIDENE CHLORIDE (VDC) IN AIR AT 200, 100, 50, 25, AND 10 PPM, 4 HOURS DAILY, 4-5 DAYS FOR 52 WEEKS. RESULTS AFTER 121 WEEKS (END OF EXPERIMENT).

| Groups nos. | Treatment | | Animals (Swiss mice 16 weeks old (Grs I,II, III,IV,V,VI) and 9 weeks old (Grs IV bis, VII) at start) | | Animals With Tumors | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Kidney adenocarcinomas | | | | Mammary Tumors[a] | | | | Pulmonary adenomas[b] | | | |
| | Concentrations | Length | Sex | No. at start | Corrected number[c] | No. | % | Average latency time (weeks)[d] | Corrected number[c] | No. | % | Average latency time (weeks)[d] | Corrected number[c] | No. | % | Average latency time (weeks)[d] |
| I | 200 ppm | 2 days | M | 60 | 1 | 0 | - | - | 6 | 0 | - | - | 5 | 0 | - | - |
| | | | F | 60 | 28 | 0 | - | - | 53 | 1 | 1.9 | 87 | 46 | 1 | 2.2 | 57 |
| | | | M and F | 120 | 29 | 0 | - | - | 59 | 1 | 1.7 | 87 | 53 | 1 | 1.9 | 57 |
| II | 100 ppm | 2 days | M | 30 | 12 | 0 | - | - | 21 | 0 | - | - | 18 | 2 | 11.1 | 62± 7 |
| | | | F | 30 | 13 | 0 | - | - | 28 | 3 | 10.7 | 46± 3 | 26 | 2 | 7.7 | 53± 2 |
| | | | M and F | 60 | 25 | 0 | - | - | 49 | 3 | 6.1 | 46± 5 | 44 | 4 | 9.1 | 58± 4 |
| III | 50 ppm | 1 week | M | 30 | 17 | 1 | 5.9 | 64 | 27 | 0 | - | - | 26 | 1 | 3.8 | 62 |
| | | | F | 30 | 14 | 0 | - | - | 28 | 2 | 7.1 | 39±13 | 27 | 3 | 11.1 | 80± 8 |
| | | | M and F | 60 | 31 | 1 | 3.2 | 64 | 55 | 2 | 3.6 | 39±13 | 53 | 4 | 7.5 | 75± 7 |
| IV | 25 ppm | 52 weeks | M | 30 | 21 | 3[e] | 14.3 | 71± 5 | 29 | 0 | - | - | 28 | 7[g] | 25.0 | 73± 6 |
| | | | F | 30 | 26 | 0 | - | - | 30 | 4[f] | 13.3 | 68±11 | 29 | 7[g] | 24.1 | 85± 6 |
| | | | M and F | 60 | 47 | 3 | 6.4 | 71± 5 | 59 | 4 | 6.8 | 68±11 | 57 | 14 | 24.6 | 30± 4 |
| IV bis | 25 ppm | 52 weeks | M | 120 | 98 | 25[e] | 25.5 | 75± 2 | 117 | 1 | 0.8 | 46 | 113 | 16[g] | 14.2 | 77± 3 |
| | | | F | 120 | 112 | 1 | 0.9 | 77 | 118 | 12[f] | 10.2 | 69± 4 | 118 | 11[g] | 9.3 | 78± 6 |
| | | | M and F | 240 | 210 | 26 | 12.4 | 75± 2 | 235 | 13 | 5.5 | 67± 4 | 231 | 27 | 11.7 | 77± 3 |
| V | 10 ppm | 52 weeks | M | 30 | 25 | 0 | - | - | 30 | 0 | - | - | 28 | 11[g] | 39.3 | 71± 5 |
| | | | F | 30 | 26 | 0 | - | - | 30 | 6[f] | 20.0 | 63± 5 | 30 | 3[g] | 16.0 | 68± 4 |
| | | | M and F | 60 | 51 | 0 | - | - | 60 | 6 | 10.0 | 63± 5 | 58 | 14 | 24.1 | 70± 4 |
| VI | No treatment (Controls) | | M | 100 | 56 | 0 | - | - | 92 | 1 | 1.1 | 25 | 80 | 3 | 3.7 | 66± 7 |
| | | | F | 100 | 73 | 0 | - | - | 97 | 2 | 2.1 | 49± 7 | 92 | 4 | 4.3 | 56± 4 |
| | | | M and F | 200 | 129 | 0 | - | - | 189 | 3 | 1.6 | 41± 9 | 172 | 7 | 4.1 | 60± 4 |
| VII | No treatment (Controls) | | M | 90 | 70 | 0 | - | - | 80 | 0 | - | - | 73 | 3 | 4.1 | 56±11 |
| | | | F | 90 | 85 | 0 | - | - | 88 | 1 | 1.1 | 83 | 86 | 2 | 2.3 | 75±12 |
| | | | M and F | 180 | 155 | 0 | - | - | 168 | 1 | 0.6 | 83 | 159 | 5 | 3.1 | 64± 8 |

[a] All mammary tumors in females were histologically diagnosed as carcinomas.

[b] Some pulmonary adenomas were cellular atypias.

[c] Alive animals when the first tumor was observed: kidney adenocarcinoma, 55 weeks; mammary tumor, 27 weeks; pulmonary adenoma, 36 weeks. The percentages refer to the corrected numbers.

[d] Average time from the start of the experiment to the detection (at the periodic control or at autopsy).

SOURCE: Maltoni et al. (1985).

[e] $p < 0.01$, combined 25 ppm (28/119) males vs. combined control males (0/196) by chi-square test. Based on corrected numbers.

[f] $p < 0.01$, combined control females (3/185) vs. 10 ppm females (6/30) and vs. combined 25 ppm females (16/148). Based on corrected numbers.

[g] $p < 0.01$, combined control females (6/153) vs. 10 ppm females (11/28) and vs. combined 25 ppm males (29/294). Also, combined control females (6/178) vs. 10 ppm females (3/30) and vs. combined 25 ppm females (18/147).

for carcinogenesis in the Maltoni et al. (1985) study. Degeneration and necrotic changes in kidneys, especially in the tubular region, were noted by Maltoni et al. (1985) in male Swiss mice that died from exposure to 200 ppm vinylidene chloride, 4 hours daily, for 2 days. These investigators observed a similar effect in the kidneys of mice that developed kidney adenocarcinomas in the lifetime inhalation study. Therefore, they recommend that further studies on animals of other species and strains would provide additional useful information.

The significant ($p < 0.01$) increase in mammary carcinomas in treated female Swiss mice would indicate evidence for the carcinogenicity of vinylidene chloride in these animals. However, Maltoni et al. (1985) concluded that for mice a direct relationship between the induction of mammary tumors, as well as pulmonary adenomas, and vinylidene chloride exposure needs further clarification.

Maltoni et al. (1985) also stated that survival of mice exposed to 10 and 25 ppm vinylidene chloride was higher than survival of the control groups. Numbers of mice surviving at 27, 36, and 55 weeks are given in Table 10-30, herein, and Maltoni et al. (1977) observed an interim survival for female mice of: (1) 47% in the 25 ppm group (IV), 20% in the 10 ppm group (V), and 21% in the matched control group (VI) when these groups were 98 weeks old, and (2) 40% in the 25 ppm group (Group IV bis) and 40% in the matched control group (VII) when these groups were 91 weeks old. Maltoni et al. (1985) presented survival data and their statistical analyses of pulmonary adenoma and mammary carcinoma data in mice to support their conclusions quoted above. Their analyses include data presented in Table 10-31 which were obtained from the Maltoni et al. (1985) report. The authors noted that "with one exception" slightly higher mean survival times for the treatment groups were not significantly ($p < 0.05$)

TABLE 10-31. STATISTICAL ANALYSES OF SURVIVAL, MAMMARY CARCINOMA INCIDENCE AND PULMONARY ADENOMA INCIDENCE FOR MALE AND FEMALE SWISS MICE IN A CARCINOGENICITY STUDY OF VINYLIDENE CHLORIDE

Mean and Standard Deviation (in weeks) of lifetime
(Test of Krauth, one-sided)

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Group[a] | Animals at start | Lifetime Mean | Lifetime Stand. Dev. | Animals at start | Lifetime Mean | Lifetime Stand. Dev. |
| VII | 90 | 70.7 C | 22.58 | 90 | 81.0 C | 18.51 |
| VI | 100 | 75.4 C | 22.08 | 100 | 81.1 C | 20.32 |
| V | 30 | 83.7 x | 15.77 | 30 | 87.5 * | 12.97 |
| IV bis | 120 | 75.0 | 15.85 | 120 | 84.3 | 17.41 |
| IV | 30 | 79.6 * | 17.03 | 30 | 90.0 x | 18.40 |

Tumor incidence for MA and PA at the end of experiment; N.TU (respectively N.MA, N.PA) is the number of animals with tumors (respectively MA, PA
(Exact Fisher Test, one-sided)

| | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| Group | Animals at start | N.TU[b] | N.MA[c] | N.PA[d] | Animals at start | N.TU | N.MA | N.PA |
| VII | 100 | 7 | 0 | 3 | 100 | 15 | 2 | 4 |
| VI | 30 | 11 x | 0 | 11 x | 30 | 15 x | 6 x | 3 |
| V | 30 | 11 x | 0 | 7 x | 30 | 17 x | 4 * | 7 x |
| IV bis | 90 | 7 | 0 | 3 | 90 | 12 | 1 | 2 |
| IV | 120 | 43 x | 1 | 16 x | 120 | 40 x | 12 x | 11 * |

Tumor incidence for MA and PA at the end of experiment; N.MA (respectively N.PA) is the number of animals with MA (respectively PA) (Logrank test)

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Group | Animals at start | N.MA | N.PA | Animals at start | N.MA | N.PA |
| VII | 100 | 0 | 3 | 100 | 2 | 4 |
| V | 30 | 0 | 11 x | 30 | 6 x | 3 |
| IV | 30 | 0 | 7 x | 30 | 4 | 7 * |
| VII | 90 | 0 | 3 | 90 | 1 | 2 |
| IV bis | 120 | 1 | 16 * | 120 | 12 * | 11 |

[a] VII - Matched control for Group IV bis, VI - Matched control for Groups V and IV, V - 10 ppm VDC, IV bis and IV - 25 ppm VDC exposure.

[b] N.TU -- Number of animals with tumors.

[c] N.MA -- Number of animals with mammary tumors.

[d] N.PA -- Number of animals with pulmonary tumors.

[C] control

[*] $p \leq 0.05$

[x] $p < 0.01$

SOURCE: Maltoni et al., 1985.

different from those of their matched controls by a rank test of Krauth. The significant ($p < 0.05$) differences shown in Table 10-31 for survival include comparisons of Groups IV and V with Group VII, which was not the matched control group for these two treatment groups, and Group IV bis with Group IV, which were the two groups exposed to 25 ppm vinylidene chloride; hence, the authors indicated that "with one exception" significant differences in mean survival were found only between groups which began their exposure at different ages.

Statistically significant ($p < 0.01$) differences in tumor incidences between control and treated mice shown in Table 10-30 were calculated using the chi-square test by combining control and 25 ppm groups since, outside of differences in age at the start of the study and the two-week difference in the starting times of the study (Table 10-26), similar tumor incidences for the separate control and 25 ppm groups were found with the same protocol. Maltoni et al. (1985) found statistically significant ($p < 0.05$) increases in mammary carcinoma and pulmonary adenoma incidences in treated mice compared to matched control mice by the Fisher exact test (Table 10-31); however, their analysis of these data by the Log Rank test, to take into account survival patterns, indicated a lower level of statistical significance for these differences between control and treated mice. A dose-related increase in tumor incidence in treated mice was not clearly apparent from the data in Table 10-31.

In summary, the studies by Maltoni et al. (1985) show clearly significant ($p < 0.01$) increases in the incidence of kidney adenocarcinomas in male Swiss mice and mammary carcinomas in female Swiss mice chronically exposed to vinylidene chloride. However, the authors concluded that the relationship between vinylidene chloride exposure and mammary carcinoma induction in female mice is difficult to evaluate since it is not dose-related. There was a significant ($p < 0.05$) increase in the incidence of fibromas and fibroadenomas in each treated group of female Sprague-Dawley rats compared to controls in the inhalation study; however, there were no significant ($p < 0.05$) increases in mammary carcinoma incidence between treated and control rats, and there was no clear dose-response for induction of mammary tumor in treated rats.

A broader evaluation of the carcinogenicity of vinylidene chloride by Maltoni et al. (1985) might have been possible if exposure periods longer than 12 months had been used and if, at the beginning of treatment, younger animals, such as weanlings, had been evaluated to cover the portion of their lifespans during growth to adulthood. It is notable that of the two groups of male mice exposed to 25 ppm vinylidene chloride, the group in which exposure was initiated at 9 weeks experienced a higher incidence of kidney adenocarcinomas than the group in which exposure initiation was delayed until 16 weeks. It does not appear that doses as high as those maximally tolerated were used in the inhalation study in hamsters and the gavage study in rats.

Viola and Caputo (1977) exposed male and female Wistar strain rats initially 2 months old in an inhalation chamber for 4 hours per day, 5 days per week, to vinylidene chloride at 200 ppm (99.8% pure) for 5 months, followed by an additional 7 months of reduced exposure to vinylidene chloride at 100 ppm to avoid toxicity. All animals were observed until death (22 to 24 months). Thirty untreated controls of each sex, 23 treated females, and 51 treated males were examined for tumors. During the exposure period, a rapidly growing mass appeared in the external ear duct of many of the animals, but on biopsy examination, only signs of an inflammatory reaction were present with no indications of dermal or epidermal neoplasia. Tumors of the abdominal cavity were present at the same incidence in both experimental (23%) and control (25%) rats. Although histology of the tumors was described, there was no mention of which organs, if any, were routinely evaluated histologically for neoplastic or pre-neoplastic changes. Furthermore, the 12-month exposure period was shorter than potential lifetime exposures for laboratory rats. In a second study using male and female Sprague-Dawley rats exposed to vinylidene chloride at 100 and 75 ppm (started in the summer of 1975) for an unspecified duration, there was again no increased tumor incidence in the experimental animals as compared with the controls. There were 30 rats in each control and 100 ppm group, and 21 females and 16 males in the 75 ppm group examined for tumors. At the time of the report, only gross tumors were enumerated; results from microscopic examination of the tissues from the Sprague-Dawley rats were to be reported later.

Lee et al. (1978), in a study primarily concerned with chronic inhalation exposure of CD-1 mice and CD rats (Charles River), initially about 2 months old, to vinyl chloride (99.8% pure), exposed a group of rats and mice (36 males and 36 females) to a single 55 ppm concentration of vinylidene chloride. Concurrent control groups exposed to inhalation of air alone consisted of 36 males and 36 females. The exposure was for 6 hours daily, 5 days per week for 12 months, at which time all surviving animals were terminated. Following sacrifice, the brain, pituitary, thyroid, respiratory tract, alimentary canal, urogenital organs, thymus, heart, liver, pancreas, spleen, mesenteric lymph nodes, and other organs with gross pathological lesions were histologically examined. The incidence of tumors observed in both rats and mice is given in Table 10-32. There was no statistically significant ($p < 0.05$, Fisher Exact Test) increase in tumors at any of the sites examined as compared with control animals. However, two treated male rats developed hemangiosarcomas, one in a mesenteric lymph node and one in the subcutaneous tissue; no such tumors occurred in controls. Hemangiosarcomas of the liver occurred in three mice

TABLE 10-32. TUMOR INCIDENCE IN RATS AND MICE EXPOSED TO VINYLIDENE CHLORIDE

| Species | Sex | Tumor type | No. tumors/No. exposed animals[a] (55 ppm vinylidene chloride) | No. tumors/No. control animals |
|---|---|---|---|---|
| CD-1 mice | M | Bronchioloalveolar adenoma | 6/35 | 1/26 |
| CD-1 mice | F | Bronchioloalveolar adenoma | 0/35 | 0/36 |
| CD-1 mice | M | Hemangiosarcoma of the liver | 2/35 | 0/26 |
| CD-1 mice | F | Hemangiosarcoma of the liver | 1/35 | 0/36 |
| CD-1 mice | M | Heptoma | 2/35 | 0/26 |
| CD-1 mice | F | Heptoma | 1/35 | 0/36 |
| CD rats | M | Hemangiosarcoma of any organ except liver | 2/36 | 0/35 |
| CD rats | F | Hemangiosarcoma of any organ except liver | 0/35 | 0/35 |

[a] None of the tumor increases were statistically significant when compared to controls.

SOURCE: Lee et al., 1978.

treated with vinylidene chloride; again, no such tumors occurred in controls. The short duration (12 months) of this study may have precluded observing tumors that have a long latency period.

In a follow-up study, Lee and coworkers (Hong et al., 1981) exposed 8 to 12 CD-1 mice of each sex and 4 to 16 CD rats of each sex, initially 2 months old, to vinylidene chloride vapors (99% pure) at a concentration of 55 ppm. The mice were exposed 6 hours/day, 5 days/week for a period of 1, 3, or 6 months (12 mice of each sex were treated for 6 months), while the rats were exposed on the same schedule for 1, 3, 6, or 10 months (16 rats of each sex were treated for 10 months). Concurrent controls exposed to inhalation of air alone consisted of 28 mice and 16 rats of each sex. All animals were observed for an additional period of 12 months following exposure. Histologic examinations were performed as described above in Lee et al. (1978). Focusing on males and females, respectively, in the groups with the longest durations of exposure, 18% and 21% of control mice, 50% and 42% of treated mice, 38% and 44% of control rats, and 79% and 56% of treated rats died before terminal sacrifice. Although tumors were observed in some exposed and control animals, the incidence of these tumors in treated animals was not significantly ($p < 0.05$) increased over control levels (Table 10-33). The small number of animals in each group weakens the ability of this study to detect a tumorigenic response, and the exposure durations were considerably less than potential lifetime exposures. A broader evaluation could have been made with additional exposure levels in each study.

Two studies have been performed for the Manufacturing Chemists Association to assess the effects of chronic inhalation and ingestion (in drinking water) of vinylidene chloride. The studies were conducted in two phases, the first for 90 days and the second for 2 years. Norris (1977) presented preli-

TABLE 10-33. TUMOR INCIDENCE IN RATS AND MICE EXPOSED TO VINYLIDENE CHLORIDE

| Species | Sex | Tumor type | No. tumors/No. exposed animals[a] (55 ppm vinylidene chloride) | No. tumors/No. control animals[a] |
|---|---|---|---|---|
| CD-1 mice | M | Hemangiosarcoma of the liver | 0/28 | 0/60 |
| CD-1 mice | F | Hemangiosarcoma of the liver | 0/28 | 1/60 |
| CD-1 mice | M | Hepatocellular tumor | 4/28 | 10/60 |
| CD-1 mice | F | Hepatocellular tumor | 0/28 | 1/60 |
| CD-1 mice | M | Hemangiosarcoma--various organs | 1/28 | 0/60 |
| CD-1 mice | M | Bronchioloalveolar tumor | 4/28 | 8/60 |
| CD-1 mice | F | Bronchioloalveolar tumor | 1/28 | 8/60 |
| CD rats | M | Hepatocellular carcinoma | 0/34 | 1/36 |
| CD rats | M | Hemangiosarcoma of the liver | 1/34 | 0/36 |
| CD rats | F | Mammary fibroadenoma | 5/36 | 5/36 |
| CD rats | F | Mammary adenocarcinoma/carcinoma | 0/36 | 1/36 |

[a] Combined incidences for all exposure periods.

SOURCE: Hong et al., 1981.

minary results of these studies with a detailed final report of the drinking water study presented by Humiston et al. (1978) and Quast et al. (1983). An additional report of these two studies to the open literature was provided by Rampy et al. (1977), and the final results of the inhalation study have been presented by McKenna et al. (1982).

In the inhalation study (Rampy et al., 1977; McKenna et al., 1982), Sprague-Dawley rats, with initial body weights of 250 to 300 g for males and 190 to 230 g for females, were exposed to vinylidene chloride (≧99% pure) for 6 hours per day, 5 days per week for a period of 18 months, and animals that survived the treatment were observed for an additional 6 months. A total of 86 males and 86 females per group were used for each exposure group plus a concurrent untreated control group. The animals were initially exposed to vinylidene choride at 10 or 40 ppm; however, after 5 weeks of exposure, an interim kill revealed no effects of exposure, and the concentrations were raised to 25 and 75 ppm, with these concentrations being used for the remainder of the experiment. Analytical and nominal atmospheric concentrations of vinylidene chloride in inhalation chambers were equivalent. The overall evaluation of toxicity included survival, body weights, organ weights, hematology, urinalysis, clinical chemistry, cytogenetics, gross pathology, and histopathology.

There were no overt signs of toxicity shown by the overall evaluation in the treated groups, and a significant ($p < 0.05$) increase in mortality was observed only in female rats exposed to 25 ppm during months 15 to 23 and 75 ppm at months 15, 17, and 21 (Tables 10-34 and 10-35). However, increases in mortality in treated groups might have been influenced by histopathologically diagnosed increases in chronic murine pneumonia incidence (number of rats with pneumonia/cumulative number of animals examined) as follows: males - 0/86

## TABLE 10-34. VINYLIDENE CHLORIDE: A CHRONIC INHALATION TOXICITY AND ONCOGENICITY STUDY IN RATS

### CUMULATIVE PERCENT MORTALITY FOR MALE RATS

| Months on Study | Exposure level | | | | | |
|---|---|---|---|---|---|---|
| | Control number dead | (% dead) | 25 ppm number dead | (% dead) | 75 ppm number dead | (% dead) |
| Number of rats alive on day 0[a] | 86 | | 85 | | 86 | |
| 1 | 1 | (1) | 3 | (4) | 1 | (1) |
| 2 | 1 | (1) | 3 | (4) | 1 | (1) |
| 3 | 1 | (1) | 3 | (4) | 2 | (2) |
| 4 | 1 | (1) | 4 | (5) | 3 | (4) |
| 5 | 1 | (1) | 4 | (5) | 3 | (4) |
| 6 | 3 | (4) | 4 | (5) | 4 | (5) |
| 7 | 4 | (5) | 4 | (5) | 4 | (5) |
| 8 | 5 | (6) | 5 | (6) | 5 | (6) |
| 9 | 5 | (6) | 5 | (6) | 5 | (6) |
| 10 | 5 | (6) | 6 | (7) | 7 | (8) |
| 11 | 6 | (7) | 6 | (7) | 8 | (9) |
| 12 | 8 | (9) | 7 | (8) | 8 | (9) |
| 13 | 9 | (11) | 8 | (9) | 9 | (11) |
| 14 | 10 | (12) | 8 | (9) | 10 | (12) |
| 15 | 14 | (16) | 8 | (9) | 12 | (14) |
| 16 | 19 | (22) | 17 | (20 | 12 | (14) |
| 17 | 23 | (27) | 18 | (21) | 16 | (19) |
| 18 | 27 | (31) | 25 | (29) | 27 | (31) |
| 19 | 32 | (37) | 36 | (42) | 37 | (43) |
| 20 | 39 | (45) | 47 | (55) | 47 | (55) |
| 21 | 49 | (57) | 50 | (59) | 56 | (65) |
| 22 | 54 | (63) | 58 | (68) | 66[d] | (77) |
| 23 | 63 | (73) | 65 | (77) | 71 | (83) |
| 24 | 72 | (84) | 72 | (85) | 74 | (86) |
| 25 | 73 | (85) | 72 | (85) | 78 | (91) |
| Terminal kill | 13 | | 13 | | 8 | |
| 30-Day Interim kill | 4 | | 4 | | 4 | |
| 6-Month Interim kill | 5 | | 5 | | 5 | |
| 12-Month Interim kill | 5 | | 5 | | 5 | |
| 26 Weeks cytogenetic kill[b] | 4 | | 3 | | 4 | |
| Total rats in study | 104 | | 103[c] | | 104 | |

---

[a] Excludes those rats in the interim kills and the cytogenetic kill.

[b] Four rats per exposure level were added one month after start of study.

[c] Includes one rat designated for cytogenetics study but not used due to death six weeks prior to cytogenetic kill.

[d] Statistically different from control data when analyzed using Fisher's Exact Probability test, $p < 0.05$.

SOURCE: McKenna et al., 1982.

## TABLE 10-35. VINYLIDENE CHLORIDE: A CHRONIC INHALATION TOXICITY AND ONCOGENICITY STUDY IN RATS

### CUMULATIVE PERCENT MORTALITY FOR FEMALE RATS

| Months on study | Exposure level |  |  |  |  |  |
|---|---|---|---|---|---|---|
|  | Control number dead | (% dead) | 25 ppm number dead | (% dead) | 75 ppm number dead | (% dead) |
| Number of rats alive on day 0[a] | 84 |  | 86 |  | 84 |  |
| 1 | 0 | (0) | 0 | (0) | 0 | (0) |
| 2 | 0 | (0) | 0 | (0) | 0 | (0) |
| 3 | 0 | (0) | 0 | (0) | 0 | (0) |
| 4 | 1 | (1) | 0 | (0) | 0 | (0) |
| 5 | 1 | (1) | 0 | (0) | 0 | (0) |
| 6 | 1 | (1) | 1 | (1) | 1 | (1) |
| 7 | 1 | (1) | 1 | (1) | 1 | (1) |
| 8 | 1 | (1) | 1 | (1) | 1 | (1) |
| 9 | 1 | (1) | 3 | (4) | 1 | (1) |
| 10 | 1 | (1) | 3 | (4) | 1 | (1) |
| 11 | 2 | (2) | 6 | (7) | 1 | (1) |
| 12 | 2 | (2) | 6 | (7) | 5 | (6) |
| 13 | 4 | (5) | 9 | (11) | 6 | (7) |
| 14 | 4 | (5) | 10 | (12) | 7 | (8) |
| 15 | 5 | (6) | 18[c] | (21) | 13[c] | (16) |
| 16 | 8 | (10) | 19[c] | (22) | 16 | (19) |
| 17 | 9 | (11) | 19[c] | (22) | 18[c] | (21) |
| 18 | 16 | (19) | 29[c] | (34) | 20 | (24) |
| 19 | 23 | (27) | 37[c] | (43) | 29 | (35) |
| 20 | 30 | (36) | 49[c] | (57) | 41 | (49) |
| 21 | 39 | (46) | 55[c] | (64) | 51[c] | (61) |
| 22 | 46 | (55) | 59[c] | (69) | 54 | (64) |
| 23 | 56 | (67) | 65 | (76) | 61 | (73) |
| 24 | 64 | (76) | 72 | (84) | 68 | (81) |
| 25 | 65 | (77) | 75 | (87) | 68 | (81) |
| Terminal kill | 19 |  | 11 |  | 16 |  |
| 30-Day Interim kill | 4 |  | 4 |  | 4 |  |
| 6-Month Interim kill | 5 |  | 5 |  | 5 |  |
| 12-Month Interim kill | 5 |  | 5 |  | 5 |  |
| 26 Weeks cytogenetic kill[b] | 4 |  | 4 |  | 4 |  |
| Total rats in study | 102 |  | 104 |  | 102 |  |

[a] Excludes those rats in the interim kills and the cytogenetic kill.

[b] Four rats per exposure level were added one month after start of study.

[c] Statistically different from control data when analyzed using Fisher's Exact Probability test, $p < 0.05$.

SOURCE: McKenna et al., 1982.

control, 26/85 low dose, 24/86 high dose; females - 9/84 control, 21/86 low dose, 22/84 high dose. Although control cages were in the inhalation chamber room during exposures, control animals were not placed in the inhalation chambers. Decreased body weights in both treated groups of male rats compared to controls are indicated in Table 10-36, but the relationship to a direct effect of vinylidene chloride appears rather uncertain in that a consistent slight but significant ($p < 0.05$) decrease is evident only in the low-dose group of males, no clear dose response is evident, and information on food consumption could have shown whether this could have affected the body weight trends shown. Body weights between control and treated female rats were similar (Table 10-37).

Treatment-related induction of non-neoplastic lesions was not apparent, with the possible exception of ovarian cysts in high-dose females [6/84 in controls; 8/86 in the low-dose group; 14/84 ($p < 0.05$) in the high-dose group]. An increase in midzonal fatty changes in the livers of treated female rats was evident [1/84 in controls; 6/86 ($p > 0.05$) in the low-dose group; 8/84, ($p < 0.05$) in the high-dose group] which may be treatment-related. However, these changes were mainly evident in females sacrificed at the end of exposure at 8 months, and only 1 high-dose female had this midzonal fatty change in liver during the 6-month post-exposure period. Furthermore, the total numbers of females with any type of fatty change in liver were as follows: 15/84 controls, 13/86 low-dose rats, and 16/84 high-dose rats.

Vinylidene chloride exposure did not result in significant ($p < 0.05$) increases in tumor incidence in this study, except for the mammary tumor data discussed below. Mammary tumor data are presented in Table 10-38 for comparison with mammary tumor data on female Sprague-Dawley rats exposed to vinylidene chloride in the inhalation study by Maltoni et al. (1985; Table 10-26).

## TABLE 10-36. VINYLIDENE CHLORIDE: A CHRONIC INHALATION TOXICITY AND ONCOGENICITY STUDY IN RATS

### MEAN BODY WEIGHT FOR MALE RATS

| Study Time | | | | |
|---|---|---|---|---|
| Month | Day | Controls | 15 ppm | 75 ppm |
| 0 | 0 | 275.82 | 276.05 | 282.56[a] |
| | 8 | 319.74 | 305.84[a] | 328.20[a] |
| | 13 | 346.77 | 340.06[a] | 353.56[a] |
| | 20 | 378.09 | 363.06[a] | 375.61 |
| 1 | 26 | 399.47 | 385.93[a] | 396.02 |
| | 39 | 428.59 | 412.48[a] | 423.88 |
| 2 | 52 | 448.09 | 431.80[a] | 447.24 |
| 3 | 79 | 489.09 | 468.71[a] | 488.15 |
| 4 | 113 | 518.47 | 500.00[a] | 518.94 |
| 5 | 140 | 536.55 | 512.86[a] | 539.54 |
| 6 | 174 | 561.78 | 528.74[a] | 549.27 |
| | 201 | 579.24 | 528.15[a] | 556.47[a] |
| | 209 | 572.32 | 528.50[a] | 552.79[a] |
| | 212 | 601.29 | 563.12[a] | 557.76[a] |
| 7 | 219 | 565.01 | 535.02[a] | 558.60 |
| 8 | 233 | 579.07 | 550.81[a] | 559.31[a] |
| 9 | 260 | 598.12 | 555.11[a] | 565.36[a] |
| 10 | 287 | 603.05 | 569.36[a] | 572.19[a] |
| 11 | 321 | 615.80 | 586.04[a] | 588.57[a] |
| 12 | 348 | 639.17 | 593.14[a] | 586.36[a] |
| 13 | 375 | 637.45 | 611.82[a] | 625.81 |
| 14 | 409 | 638.81 | 613.47 | 639.74 |
| 15 | 436 | 638.46 | 623.26 | 645.81 |
| 16 | 463 | 631.71 | 618.00 | 635.24 |
| 17 | 497 | 659.41 | 614.94[a] | 628.62[a] |
| 18 | 524 | 627.18 | 585.60[a] | 618.39 |
| 19 | 551 | 606.18 | 596.84 | 583.75 |
| 20 | 578 | 609.37 | 575.20 | 585.84 |
| 21 | 612 | 607.78 | 582.11 | 563.10[a] |
| 22 | 646 | 597.50 | 569.04 | 541.85 |
| 23 | 677 | 574.96 | 554.65 | 536.13 |
| 24 | 704 | 587.27 | 549.69 | 529.55 |

[a]Significantly different from control mean, $p < 0.05$.

SOURCE: McKenna et al., 1982.

## TABLE 10-37. VINYLIDENE CHLORIDE: A CHRONIC INHALATION TOXICITY AND ONCOGENICITY STUDY IN RATS

### MEAN BODY WEIGHT FOR FEMALE RATS

| Study Time | | | | |
|---|---|---|---|---|
| Month | Day | Controls | 25 ppm | 75 ppm |
| 0 | 0 | 210.24 | 212.58 | 203.22[a] |
| | 8 | 230.20 | 221.38[a] | 241.57[a] |
| | 13 | 238.48 | 243.40[a] | 247.40[a] |
| | 20 | 252.87 | 246.59[a] | 255.68 |
| 1 | 26 | 259.52 | 258.60 | 264.34 |
| | 39 | 269.89 | 277.79[a] | 280.79[a] |
| 2 | 52 | 278.78 | 280.84 | 289.26[a] |
| 3 | 79 | 294.14 | 301.51[a] | 308.79[a] |
| 4 | 113 | 311.28 | 317.23 | 325.10[a] |
| 5 | 140 | 322.03 | 329.17 | 335.96[a] |
| 6 | 174 | 332.67 | 342.63[a] | 342.12[a] |
| | 201 | 336.18 | 331.11 | 339.93 |
| | 209 | 337.11 | 334.58 | 336.71 |
| | 212 | 332.63 | 335.27 | 343.73[a] |
| 7 | 219 | 331.73 | 335.80 | 340.29 |
| 8 | 233 | 338.73 | 338.93 | 340.23 |
| 9 | 260 | 339.60 | 348.01 | 348.26 |
| 10 | 287 | 343.67 | 362.06[a] | 356.57 |
| 11 | 321 | 356.89 | 370.98[a] | 368.90[a] |
| 12 | 348 | 367.94 | 377.14 | 363.14 |
| 13 | 375 | 376.74 | 375.25 | 387.52 |
| 14 | 409 | 379.60 | 386.23 | 397.57[a] |
| 15 | 436 | 386.38 | 391.36 | 402.62 |
| 16 | 463 | 390.53 | 397.31 | 397.10 |
| 17 | 497 | 406.30 | 411.39 | 419.64 |
| 18 | 524 | 396.26 | 419.26 | 426.13 |
| 19 | 551 | 395.10 | 429.48 | 421.21 |
| 20 | 578 | 414.86 | 405.68 | 413.13 |
| 21 | 612 | 443.06 | 411.72 | 448.65 |
| 22 | 646 | 412.23 | 435.19 | 457.27 |
| 23 | 677 | 420.33 | 460.71 | 469.91 |
| 24 | 704 | 427.00 | 462.67 | 438.62 |

[a] Significantly different from control mean, $p < 0.05$.

SOURCE: McKenna et al., 1982.

## TABLE 10-38. VINYLIDENE CHLORIDE: A CHRONIC INHALATION TOXICITY AND ONCOGENICITY STUDY IN RATS

### HISTOPATHOLOGIC DIAGNOSIS AND NUMBER OF TUMORS IN FEMALE RATS

| | | 0-6 Months | 7-12 Months | 13-18 Months | 19-24 Months | Terminal kill | Cumulative results |
|---|---|---|---|---|---|---|---|
| Number of rats necropsied during the time period indicated | Control | 1 | 1 | 14 | 49 | 19 | 85 |
| | 25 ppm | 1 | 5 | 23 | 46 | 11 | 86 |
| | 75 ppm | 1 | 4 | 15 | 48 | 16 | 85 |
| **Mammary gland** | | | | | | | |
| Fibroadenoma/adenofibroma S-25 | Control | 0 | 0 | 17/10 | 78/39 | 37/15 | 132/64[b] |
| | 25 ppm | 0 | 2/2 | 40/20 | 76/38 | 16/8 | 134/68[b] |
| | 75 ppm | 1/1 | 6/4 | 17/14 | 100/40 | 33/15 | 157/74[b] |
| Fibroma S-26 | Control | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 ppm | 0 | 0 | 0 | 0 | 0 | 0 |
| | 75 ppm | 0 | 0 | 0 | 1/1 | 1/1 | 2/2[b] |
| Adenocarcinoma with pulmonary metastasis S-40 | Control | 0 | 0 | 0 | 1/1 | 0 | 1/1[b] |
| | 25 ppm | 0 | 0 | 0 | 0 | 0 | 0 |
| | 75 ppm | 0 | 0 | 0 | 0 | 0 | 0 |
| Adenocarcinoma without metastasis S-27 | Control | 0 | 0 | 0 | 1/1 | 0 | 1/1[b] |
| | 25 ppm | 0 | 1/1 | 2/2 | 1/1 | 4/3 | 8/7[a,b] |
| | 75 ppm | 0 | 0 | 2/2 | 0 | 2/2 | 4/4[b] |
| Leiomyosarcoma without metastasis S-41 | Control | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 ppm | 0 | 0 | 0 | 1/1 | 0 | 1/1[b] |
| | 75 ppm | 0 | 0 | 0 | 0 | 0 | 0 |
| Carcinosarcoma of the mammary gland S-43 | Control | 0 | 0 | 0 | 0 | 1/1 | 1/1[b] |
| | 25 ppm | 0 | 0 | 0 | 0 | 0 | 0 |
| | 75 ppm | 0 | 0 | 0 | 0 | 0 | 0 |
| Adenofibroma based on gross examination only S-44 | Control | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 ppm | 0 | 0 | 0 | 0 | 0 | 0 |
| | 75 ppm | 0 | 0 | 0 | 1/1 | 0 | 1/1[b] |
| Total number of animals with a subcutaneous and/or mammary gland tumor[c] S-02, S-05, S-25, S-26, S-27, S-40, S-41, S-43, S-44 | Control | 0 | 0 | 10 | 39 | 16 | 65 |
| | 25 ppm | 0 | 3 | 20 | 38 | 9 | 70 |
| | 75 ppm | 1 | 4 | 14 | 41 | 15 | 75[a] |
| Number of rats with a malignant tumor in mammary region or skin[c] S-05, S-27, S-40, S-41, S-43 | Control | 0 | 0 | 0 | 2 | 2 | 4 |
| | 25 ppm | 0 | 1 | 2 | 2 | 3 | 8 |
| | 75 ppm | 0 | 0 | 2 | 0 | 2 | 4 |

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[a] Significantly different from control data when analyzed using Fisher's Exact Probability Test, $p < 0.05$.

[b] Number of tumors/number of animals with tumors.

[c] A subcutaneous fibroma was found in one female in the 75 ppm group, and a subcutaneous fibrosarcoma without metastasis was observed in one female in the control group.

SOURCE: McKenna et al., 1982.

The incidence of adenocarcinoma without metastasis was significantly ($p < 0.05$) increased in low-dose females compared to controls; however, mammary adenocarcinoma incidence was not significantly ($p < 0.05$) increased in high-dose females, and adenocarcinoma without metastasis was found in another control female, which causes the adenocarcinoma incidence in low-dose females to lose significance ($p > 0.05$). The significant increase ($p < 0.05$) in total mammary tumor incidence in high-dose females compared to control females includes animals with both benign and malignant tumors and, according to the authors, total mammary tumor incidence in the control group and in each treatment group is within the historical control range for the Sprague-Dawley strain of rats at the investigating laboratory. Thus, demonstration of an induction of mammary tumor formation in female Sprague-Dawley rats under the conditions of the study by McKenna et al. (1982) does not seem apparent. Overt toxicity of vinylidene chloride in Sprague-Dawley rats does not appear evident, and an attempt to use higher exposure levels in this study might have provided a broader evaluation for carcinogenicity.

In the reports of Quast et al. (1983) and Humiston et al. (1978), male and female Sprague-Dawley rats, initially 6 to 7 weeks old, were exposed to vinylidene chloride at 50, 100, and 200 ppm (99.5% pure) in drinking water for 2 years. Because of the volatile nature of vinylidene chloride, the water was made up at higher concentrations, and the above figures reflect the average concentrations during 24-hour periods. The actual vinylidene chloride levels measured in the drinking water were $68 \pm 21$, $99 \pm 22$, and $206 \pm 33$ ppm (mean ± SD). When taking into account the water consumption of the rats, the time-weighted average dose was equivalent to 7, 10, or 20, and 9, 14, or 30 mg/kg/day in males and females, respectively. The vinylidene chloride used in this study was distilled prior to making up the test water in order to reduce to 1 to 5 ppm the concentration of the inhibitor monomethyl ether of hydroquinone (this inhibitor would normally be at low levels in copolymers used for food packaging applications). Each treatment group consisted of 48 rats of each sex, and an untreated control group consisted of 80 males and 80 females. Animals were evaluated for survival, body weight, food and water consumption, hematology, clinical chemistry, urinalysis, gross pathology, and histopathology. The animals were observed until morbidity, or 24 months, at which time surviving animals were sacrificed. The specific tissues examined are presented in Table 10-39. There was no evidence of statistically significant ($p < 0.05$) treatment-related effects of vinylidene chloride on the toxicologic end points evaluated in treated animals as compared to controls. At least 50% of each group survived for approximately as long as 20 months.

A number of neoplastic lesions were observed in both control and experimental animals. The total tumor incidence is given in Table 10-40. The tumor frequency at specific sites, as reported by Quast et al. (1983), did not show a treatment-related effect of vinylidene chloride. The only statistically ($p < 0.05$) significant increase in a specific neoplasm was in female rats exposed to vinylidene chloride at 50 ppm. These animals had an increased incidence of mammary gland fibroadenomas/adenofibromas (40/48 50 ppm females vs. 53/80 control females). This tumor was not considered by the authors to be treatment-related, since there was no dose relationship between tumor incidence, i.e., 36/48 100 ppm females and 35/48 200 ppm females, and exposure to vinylidene chloride. Also, the spontaneous incidence of mammary tumors was high in this strain of rats, and the tumor incidence observed in the 50 ppm females was within the limits for historical controls at the testing laboratory. Four control, 1 low-dose, and 2 mid-dose females had mammary carcinomas. No treatment-related effects of vinylidene chloride on non-neoplastic lesions were

## TABLE 10-39. REPRESENTATIVE TISSUE SPECIMENS OBTAINED AT NECROPSY FROM ALL ANIMALS

esophagus
salivary glands
stomach
large intestine
pancreas
liver
kidneys
urinary bladder
prostate
accessory sex glands
epididymides
testes
ovaries
uterus
mammary tissue (females)
brain (cerebrum, cerebellum with brain stem)
pituitary gland
spinal cord
peripheral nerve
trachea
lungs (bronchi)
nasal turbinates
sternum and sternal bone marrow[a]
spleen
mediastinal lymphoid tissue[b] (thymus, mediastinal lymph nodes)
lymph nodes (mesenteric)
heart
aorta
skeletal muscle
adrenal glands
thyroid gland
parathyroid gland[b]
adipose tissue
skin
any gross lesion or mass
eyes[c]

---

[a] Sternum and enclosed bone marrow were to be evaluated histologically only if indicated by abnormal findings in the hematological studies.

[b] These tissues were evaluated histologically only to the extent that they were included in the routine sections of adjacent larger organs.

[c] Eyes were saved in formalin if the rat died or was culled from the study. If the rat was from the terminal kill, a portion of these were fixed in Zenker's fixative and the remainder in formalin. Only selected animals had their eyes microscopically examined.

SOURCE: Humiston et al., 1978.

## TABLE 10-40. TUMOR INCIDENCE FOLLOWING INGESTION OF VINYLIDENE CHLORIDE (VDC)

| Concentration of VDC in water (ppm) | Total number of neoplasms | | Total number of rats in group | | Average number of neoplasms/number of rats | |
|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female |
| 0 | 100 | 187 | 80 | 80 | 1.3 | 2.3 |
| 50 | 53 | 132 | 48 | 48 | 1.1 | 2.8 |
| 100 | 43 | 120 | 48 | 48 | 0.9 | 2.5 |
| 200 | 53 | 124 | 47 | 48 | 1.1 | 2.6 |

SOURCE: Humiston et al., 1978.

evident, except for minimal fatty change and swelling in the livers of high-dose males and females in all treatment groups. The dose of vinylidene chloride ingested in the drinking water appeared low enough to produce no overt toxic effects. The use of higher exposure levels might have provided a stronger evaluation of the carcinogenic potential of vinylidene chloride; however, the authors indicated that 200 ppm, the maximum concentration of vinylidene chloride in water used in this study, was the highest concentration possible given the solubility of vinylidene chloride (2.25 g/L at 25°C).

The National Cancer Institute/National Toxicology Program (NCI/NTP, 1982) has prepared a report on a cancer bioassay of vinylidene chloride (99% pure) performed on Fischer 344 rats and B6C3F1 mice, initially 9 weeks old. Male and female rats (50 animals of each sex) received vinylidene chloride, dissolved in corn oil, by gavage at doses of 1 and 5 mg/kg, while male and female mice (50 animals of each sex) received doses of 2 and 10 mg/kg. The animals were treated 5 days/week for a total of 104 weeks. Animals were observed for gross signs of toxicity during the exposure period, as indicated by food consumption patterns, body weight gain, and mortality. The treated and control animals were killed after 104 weeks of exposure or when moribund, and the tissues indicated in Table 10-41 were examined histologically for both tumorigenic and non-tumorigenic pathology.

TABLE 10-41. TISSUES EXAMINED[a] FOR HISTOLOGIC CHANGES IN THE NCI BIOASSAY

---

skin
lungs and bronchi
trachea
bone and bone marrow
spleen
lymph nodes
heart
salivary gland
adrenal
thyroid
parathyroid
seminal vesicles (male)
testis (male)
thymus
esophagus
liver
pancreas
stomach
small intestine
large intestine
kidney
urinary bladder
pituitary
mammary gland
prostate
brain
uterus (female)
ovary (female)
larynx

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[a] These tissues were examined in all animals, except where advanced autolysis or cannibalism prevented meaningful evaluation.

SOURCE: NCI/NTP, 1982.

The survival (greater than 50% for the whole study for all groups) and weight gain of rats were unaffected by treatment with vinylidene chloride; however, 12 control and 10 low-dose male animals were accidentally killed as a result of a 5-hour exposure to 37°C on week 82 of the study. Including the male rats accidentally killed, survival of rats at terminal sacrifice was as follows: 20/50 vehicle control males, 24/50 low-dose males, 37/50 high-dose males, 27/50 vehicle control females, 28/50 low-dose females, and 29/50 high-dose females. In mice, only survival in the low-dose female group was adversely affected by treatment. However, at least 64% of the mice in all groups survived the study (33/50 vehicle control males, 35/50 low-dose males, 36/50 high-dose males, 40/50 vehicle control females, 32/50 low-dose females, and 42/50 high-dose females). Body weight gain was slightly depressed in both dose groups of male mice and in female mice of the low-dose group as compared to either the corresponding high-dose or control animals (Table 10-42); however, a dose-response was not evident, and body weight decreases were greater in low-dose than in high-dose mice. Dose-related non-neoplastic lesions consisted of liver necrosis in mice (male controls, 1/46, 2%; low-dose, 3/46, 7%; high-dose, 7/49, 14%; female controls, 0/47, 0%; low-dose, 4/49, 8%; high-dose, 1/49, 2%) and inflammation of the kidney in rats (male controls, 26/49, 53%; low-dose, 24/48, 50%; high-dose, 43/48, 90%; female controls, 3/49, 6%; low-dose, 6/49, 12%; high-dose, 9/49, 20%). It is noted in the NCI/NTP (1982) report that chronic nephritis is a common lesion in aging rats. Other histopathologic changes of a non-tumorigenic type were observed randomly in control and treated animals, and were not considered treatment-related.

In rats and mice, increased tumor incidences were observed in a number of organs (Table 10-43). While unadjusted analyses of these data suggested that some responses were marginally statistically significant at the $p = 0.05$ level, such analyses do not take into account the multiple organs compared. As indicated by NCI/NTP (1980) and discussed in greater detail by Gart et al. (1979), it is more accurate to account for multiplicity of comparisons by the Bonferroni correction when using the Fisher exact test, and to account for differing patterns of mortality among the dose groups by time-adjusted analyses. Making these corrections, the only statistically significant ($p < 0.05$) increased response was for lymphomas in the low-dose group female mice compared to the matched controls using the Fisher exact test. Furthermore, only

TABLE 10-42. MEAN BODY WEIGHT CHANGE (RELATIVE TO CONTROLS) OF MICE ADMINISTERED VINYLIDENE CHLORIDE BY GAVAGE

| | | Mean body weight change grams | | | Weight change relative to controls[a] (percent) | |
|---|---|---|---|---|---|---|
| | Week No. | Control | Low dose | High dose | Low dose | High dose |
| Male mice | 0 | 28[b] | 24[b] | 25[b] | | |
| | 1 | 1 | 2 | 2 | +100 | +100 |
| | 20 | 13 | 11 | 13 | -15 | 0 |
| | 40 | 19 | 17 | 19 | -20 | 0 |
| | 60 | 22 | 21 | 23 | -5 | +5 |
| | 80 | 21 | 20 | 22 | -5 | +5 |
| | 100 | 20 | 21 | 21 | +5 | +5 |
| Female mice | 0 | 19[b] | 18[b] | 18[b] | | |
| | 1 | 2 | 2 | 3 | 0 | +50 |
| | 20 | 9 | 8 | 9 | -11 | 0 |
| | 40 | 12 | 12 | 13 | 0 | +8 |
| | 60 | 17 | 15 | 18 | -12 | +6 |
| | 80 | 19 | 18 | 14 | -5 | +35 |
| | 100 | 24 | 21 | 24 | -13 | 0 |

[a] Weight change relative to controls =

$$\frac{\text{Weight change (dosed group) - Weight change (control group)}}{\text{Weight change (control group)}} \times 100$$

[b] Initial weight

SOURCE: NCI/NTP, 1982.

TABLE 10-43. TUMORS WITH INCREASED INCIDENCE IN RATS AND MICE, AS INDICATED BY THE FISHER EXACT TEST OR THE COCHRAN-ARMITAGE TEST FOR LINEAR TREND

| Species | Tumor | Control | Incidence | |
|---|---|---|---|---|
| | | | Low Dose | High Dose |
| rat (male) | adrenal, pheochromocytomas | 6/50 (12%)<br>(p=0.01)[a] | 5/48 (10%) | 13/47 (28%)<br>(p=0.045)[b] |
| rat (male) | pancreatic islet-cell, adenomas/carcinoma[c] | 4/49 (8%)<br>(p=0.025)[a] | 1/47 (2%) | 8/48 (17%) |
| rat (male) | testes, interstitial-cell | 43/50 (86%)<br>(p=0.013)[a] | 39/47 (83%) | 47/48 (98%)<br>(p=0.034)[b] |
| rat (female) | pituitary, adenomas | 16/48 (33%)<br>(p=0.017) | 20/49 (41%) | 24/43 (56%)<br>(p=0.026)[b] |
| rat (male) | subcutaneous, fibromas | 0/50 (0%)<br>(p=0.024)[a] | 1/48 (2%) | 4/48 (8%) |
| mice (female) | lymphomas | 2/48 (4%) | 9/48 (18%)<br>(p=0.028)[b] | 6/50 (12%) |

[a] Significant for linear trend by Cochran-Armitage test ($p < 0.05$).

[b] Significant by Fisher exact test ($p < 0.05$).

[c] All pancreatic tumors were carcinomas, except for adenomas in two high-dose males. Carcinomas alone were not significant by the Cochran-Armitage test.

SOURCE: NCI/NTP, 1982.

lymphomas represent a statistical increase in malignant tumors in Table 10-43. However, a similar increase in the high-dose group was not noted, and the Cochran-Armitage test for linear trends was not significant. Since the matched controls had a low incidence (4%) of lymphomas when compared to the incidence (9.8%) in historical controls, this effect was not considered to be treatment-related. Thus, the conclusion in the NCI/NTP (1982) report is that vinylidene chloride was not carcinogenic to F344 rats or B6C3F1 mice under the conditions of this assay. Survival and body weight data suggest that higher doses of vinylidene chloride could have been given to rats and mice to more strongly challenge these animals for carcinogenicity.

Vinylidene chloride was tested for its potential as a brain carcinogen on the basis that vinyl chloride causes neuroblastomas in animal studies and is associated with brain neoplasms in exposed humans (Maltoni et al., 1982). No increase in the incidence of gliomas, meningiomas, or ependymomas was reported among Sprague-Dawley rats exposed by inhalation to vinylidene chloride at 10, 25, 50, 100, or 150 ppm for 4 or 5 days weekly for 52 weeks. Male and female rats exposed by ingestion (gavage) to 0.5, 5, 10, or 20 mg/kg of vinylidene chloride for 5 days weekly for 52-59 weeks did not show an increased incidence of brain tumors when compared to controls.

Ponomarkov and Tomatis (1980) assessed the carcinogenic potential of vinylidene chloride in BDIV rats exposed to the compound starting in utero and continuing to 120 weeks of age. Pregnant rats (24 animals) received a single 150 mg/kg dose of vinylidene chloride (99% pure) by gavage in corn oil on day 17 of gestation. Following birth, the pups (89 males and 90 females) received vinylidene chloride by gavage at a dose of 50 mg/kg weekly for the duration of the study. The animals were killed at the termination of the study or when moribund, and major organs and gross lesions were examined histologically.

There was no treatment-related effect on the reproductive success of the dams or on the survival or weight gain of the offspring of rats treated with vinylidene chloride. Non-tumorigenic lesions in the liver, consisting of degeneration of the parenchymal cells, liver necrosis, and hemorrhage, were observed in treated animals moribund after 80 weeks of treatment or killed at termination. In cases of early death (30 weeks of treatment), lung and kidney congestion were also observed in treated rats. The total tumor incidence in either the dams or the offspring was not significantly increased by treatment (Table 10-44); however, there was an increased incidence of meningiomas in male rats, although this was also not significantly different from control levels. Although slight differences in tumor incidence were observed following vinylidene chloride treatment, this study did not demonstrate a statistically significant effect of vinylidene chloride treatment on the induction of tumors in either the dams or their offspring. Although liver lesions were seen, it appears that higher doses could possibly have been used, since no effect was observed on survival and body weight.

Skin application has been used to assay vinylidene chloride for tumor initiation and complete carcinogen action in lifetime studies using the two-stage tumorigenesis model in female Ha:ICR Swiss mice (Van Duuren et al., 1979). In two-stage tumorigenesis, an initiator agent is applied in a single dose to the skin of a mouse. The initiator does not produce tumors at the applied concentration, but predisposes the skin so that later repeated applications of a promotor (an agent that by itself will not produce tumors) will cause the formation of tumors. A complete carcinogen is one which, if applied in sufficient concentrations, can produce tumors by itself. When vinylidene chloride was applied three times each week at a dose of 121 mg/mouse to the shaved backs of 30 mice, no tumors were observed; however, when a single dose

## TABLE 10-44. TUMOR INCIDENCE IN FEMALE BDIV RATS TREATED WITH VINYLIDENE CHLORIDE (VDC) IN THEIR PROGENY TREATED WEEKLY FOR LIFE AND CONTROLS

| Group | Effective number of rats[a] | Tumor bearing rats | | Number of tumors | | Animals with more than one tumor | |
|---|---|---|---|---|---|---|---|
| | | n | % | Total | per rat | n | % |
| Females given VDC | 23 | 11 | 47.8 | 14 | 0.6 | 3 | 13 |
| Progeny treated weekly with VDC | | | | | | | |
| Males | 81 | 31 | 38.3 | 35 | 0.4 | 4 | 4.9 |
| Females | 90 | 53 | 66.3 | 64 | 0.8 | 11 | 13.8 |
| Females given olive oil | 14 | 5 | 37.7 | 7 | 0.5 | 2 | 14.3 |
| Progeny treated weekly with olive oil | | | | | | | |
| Males | 49 | 16 | 32.7 | 16 | 0.3 | - | - |
| Females | 47 | 24 | 51.1 | 29 | 0.6 | 5 | 10.6 |

| Group | Meninges | | Oral cavity | | Stomach | | Liver | | Soft tissue | | Mammary gland | | Ovary | | Other | | Liver nodules | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % |
| Females given VDC | - | - | 2 | 8.7 | 1 | 4.3 | - | - | - | - | 8 | 34.8 | 2 | 8.7 | 1[b] | 4.3 | 2 | 8.7 |
| Progeny treated weekly with VDC | | | | | | | | | | | | | | | | | | |
| Males | 6 | 7.4 | 5 | 6.2 | 1 | 1.2 | 1 | 1.2 | 9 | 11.1 | 1 | 1.2 | - | - | 12[c] | 14.8 | 2 | 2.5 |
| Females | - | - | 1 | 1.3 | 2 | 2.5 | 3 | 3.8 | 7 | 8.8 | 39 | 48.8 | 6 | 7.5 | 6[d] | 7.5 | 6 | 7.5 |
| Females given olive oil | - | - | 1 | 7.1 | - | - | - | - | 1 | 7.1 | 4 | 28.6 | - | - | 1[e] | 7.1 | - | - |
| Progeny treated weekly with olive oil | | | | | | | | | | | | | | | | | | |
| Males | 1 | 2.0 | 2 | 4.1 | - | - | - | - | 4 | 8.2 | - | - | - | - | 9[f] | 18.4 | - | - |
| Females | - | - | 1 | 2.1 | 1 | 2.1 | - | - | - | - | 22 | 46.8 | 3 | 6.4 | 2[g] | 4.3 | - | - |

[a] The percentage and the number of tumors per rat are expressed in relation to the effective number of rats.

[b] Survivors at the time the first tumors were observed.

[c] Urinary bladder papilloma; 1 lymphoma, 4 pituitary adenomas; 3 adrenal cortical adenomas; 1 spleen haemangioma; 1 lung sarcoma, pleomorphic; 1 skin squamous cell carcinoma; 1 seminoma.

[d] 1 salivary gland carcinoma; 1 salivary gland adenoma; 1 lymphoma; 1 pituitary adenoma; 1 rectal adenomalous polyp; 1 uterine adenoma.

[e] Adrenal cortical adenoma.

[f] 1 osteosarcoma; 1 mediastinal sarcoma; 1 lung epidermoid carcinoma; 2 lymphomas; 1 spleen haemangioma; 2 pituitary adenomas; 1 adrenal cortical adenoma.

[g] 1 lymphoma; 1 uterine adenoma.

SOURCE: Ponomarkov and Tomatis, 1980.

of 121 mg/mouse of vinylidene chloride was applied to the skin of 30 mice, followed by repeated application of phorbol myristate acetate (PMA) as the promotor, a significant increase ($p < 0.005$) in skin papillomas (8 mice with papillomas/9 total papillomas) was observed as compared to controls. No skin papillomas were observed in 30 controls treated with acetone, the dosing vehicle, and 100 untreated controls, whereas 9 mice (10 total papillomas) of 120 mice given 0.0025 mg PMA and 6 mice (7 total papillomas) of 90 mice given 0.005 mg PMA on the same schedule as that for treated mice were found. Squamous cell carcinomas on the skin in the initiation-promotion study were observed on one mouse in the vinylidene chloride group, one mouse in the low-dose PMA group, and 2 mice in the high-dose PMA group. A positive control group of 30 mice in the initiation-promotion study given 0.02 mg/mouse of 7,12-dimethylbenz[a]anthracene as the initiating agent developed papillomas in 29 mice (317 total papillomas) and local squamous cell carcinomas in 14 mice. In the same study, vinylidene chloride was also tested for complete carcinogenic action in 30 female Swiss ICR/Ha mice by repeated subcutaneous injection once a week at a dose of 2 mg/mouse. Following treatment for 548 days, no tumors were observed at the site of injection or at sites distant from the site of injection. Although this study indicates that vinylidene chloride is a tumor initiator in mouse skin, it is not clear how this relates to the processes of complete chemical carcinogenesis in other organs, or if tumor initiation as observed here is a phenomenon solely restricted to the skin of mice. The relevance of positive results in this tumor initiation study with regard to the assessment of human health effects is not clear, particularly since complete carcinogenic activity could not be demonstrated. Maximally tolerated doses, estimated from preliminary short-term tests, were used in the tests for carcinogenicity performed by Van Duuren et al. (1979).

In summary, the evidence supporting the carcinogenicity of vinylidene chloride is limited. It has been shown that vinylidene chloride is a mutagen in bacterial and yeast assay systems, although the evidence is limited for classifying vinylidene chloride as a human mutagen. Van Duuren et al. (1979) have demonstrated that vinylidene chloride acts as a tumor initiator in mouse skin; however, of the three animal species used to assess the carcinogenicity of this compound--rats, mice, and hamsters--only one strain of mice developed a unique tumor type (kidney carcinomas) following exposure to vinylidene chloride. Maltoni (1977) and Norris (1982) suggested that the observed increase in kidney tumors may be a species- and strain-specific effect. Maltoni (1977), Maltoni et al. (1977), and Maltoni and Patella (1983) suggested a relationship between the sensitivity to acute toxic effects of vinylidene chloride and carcinogenic responses in the strains of rats and mice evaluated for vinylidene chloride carcinogenicity in their studies. A statistically significant increase in mammary carcinomas was found in female Swiss mice by Maltoni et al. (1985), but these investigators concluded that this evidence was not conclusive and requires further study.

There have been a number of other carcinogenicity bioassays of vinylidene chloride in which no statistically significant increase in tumor incidence was observed (Table 10-23). From these studies, it could be concluded that vinylidene chloride has not been found to be significantly carcinogenic in rats or hamsters, nor in mice when administered by the oral route. The only published inhalation studies in mice, except for the positive bioassay of Maltoni et al. (1985), used either a shorter exposure period (Hong et al., 1981) or a shorter observation period (Lee et al., 1978), although the level of exposure in both studies was higher (55 ppm, 6 hours/day) than the high-dose group (25 ppm for 4 hours/day) used by Maltoni et al. (1985). Furthermore, higher doses possibly could have been tested in several of the negative studies. Nonetheless, in view of the present evidence for vinylidene chloride carcinogenicity found in one mouse strain in the inhalation study by Maltoni et al. (1977, 1985), the available evidence regarding the carcinogenicity of vinylidene chloride in animals is thus seen to be limited and not sufficient for a firm conclusion on the potential carcinogenicity of this compound in humans.

### 10.5.2 Epidemiologic Studies

Adequate data regarding the carcinogenic potential of vinylidene chloride in humans are lacking. One study, that of Ott et al. (1976) investigated 138 Dow Chemical Company workers exposed primarily to vinylidene chloride, and gave mortality data and the results of health examinations. The Ott et al. (1976) study is a historic prospective cohort study of 138 employees whose first employment extends back to 1940. However, most began their employment after 1950: 74 in the period from 1950 to 1959, and 55 from 1960 to 1969. The cut-off date for this study was January 1, 1974. The authors noted that vinyl chloride is a common contaminant of vinylidene chloride monomer and that liquid vinylidene chloride contained less than 0.2% by weight of vinyl chloride in recent years prior to publication of their study. In this study, time-weighted average (TWA) exposures were estimated based on job descriptions and industrial hygiene surveys. Based on TWA exposure estimates, each job initially fell into one of four categories: <10 ppm, 10 to 24 ppm, 25 to 49 ppm, and 50+ ppm. However, since operators in fiber production were the only workers with exposures above 25 ppm TWA, only three concentration categories were used to calculate cumulative dose: <10 ppm (5 ppm used for calculation); 10 to 24 ppm (17 ppm used for calculation); and ≥25 ppm (43 ppm used for calculation). Cumulative career exposures were estimated by multiplying the TWA times the duration, in months, of potential exposure (Table 10-45). As indicated in the table, the size of the population having a lengthy duration of exposure or a long latency period since the initial time of exposure was small. Moreover, it cannot be determined from the data presented in the study report whether those individuals for whom the greatest time had elapsed since initial exposure were also among the most heavily exposed subjects. Thus, it is possible that for detection of long-latency diseases such as cancer, the population examined in this study may not be adequate.

TABLE 10-45. ESTIMATED CUMULATIVE DOSE, DURATION OF EXPOSURE AND DATE OF FIRST EXPOSURE AMONG 138 INDIVIDUALS EXPOSED TO VINYLIDENE CHLORIDE

| Exposure measures | Total population |
|---|---|
| Estimated career dosage (TWA x months of exposure) | |
| <500 ppm months | 50 |
| 500-999 ppm months | 28 |
| 1000-1999 ppm months | 28 |
| 2000+ ppm months | 32 |
| Duration of exposure | |
| <12 months | 35 |
| 12-59 months | 43 |
| 60-119 months | 35 |
| 120+ months | 25 |
| Date of first exposure | |
| 1940-1949 | 9 |
| 1950-1959 | 74 |
| 1960-1969 | 55 |

[a] Status as of January 1974.

SOURCE: Ott et al., 1976.

Cohort mortality was compared with U.S. white male mortality for 1942, 1947, 1952, 1957, 1962, 1967, and 1971 by the indirect method. The most recent health inventory for the cohort was compared with matched controls. For the total cohort of 138 persons, 5 deaths were recorded versus 7.5 expected (Table 10-46). One death of an individual with a 745 ppm cumulative dose was attributed to malignancy (versus 1.1 expected) and this was due to respiratory cancer; however, his smoking history was not known. The expected respiratory cancer death rate was 0.3 for the total cohort; 0.2 for the total cohort 15+ years after first exposure to vinylidene chloride; and 0.2 for the cohort with 500+ ppm months of exposure (Table 10-46). An examination of company health inventory records on this same cohort revealed no statistically significant ($p < 0.05$) clinical difference between the vinylidene chloride-exposed group and matched (for age and smoking) controls. Although duration of employment appears sufficient to have provided an opportunity for exposure to vinylidene chloride, yet, in order to observe a statistically significant elevated risk of cancer at probability level $p < 0.05$ with 80% power, a minimum of 12 cancer deaths would have had to occur (relative risk of 10.6) given that the author expected only 1.1 in his small cohort of 138 white males without regard for latency. If one assumes a 10-year latent period for the development of cancer, the number of person-years of observation would be reduced by approximately 50%, and consequently the power to detect a significant relative risk of even 10.6 would subsequently decline from 80% to 43% in that segment of the cohort where the occurrence of cancer could most credibly be attributable to exposure to vinylidene chloride. In order to maintain a power of 80%, the relative risk needed to detect a significant difference at the $p < 0.05$ level is about 16 based upon a CAG estimate of 0.6 cancer deaths. Relative risks of such magnitude occur generally only with exposures to exceptionally strong carcinogens

## TABLE 10-46. OBSERVED AND EXPECTED DEATHS AMONG VINYLIDENE CHLORIDE EMPLOYEES BY CAUSE 1945-1973

| | Total cohort | | Total cohort 15+ years after first exposure | |
|---|---|---|---|---|
| | Observed | Expected | Observed | Expected |
| All causes | 5 | 7.5 | 2 | 2.6 |
| Total malignancies | 1 | 1.1 | 1 | 0.5 |
| Digestive cancer | | 0.3 | | 0.1 |
| Respiratory cancer | 1 | 0.3 | 1 | 0.2 |
| All other | | 0.5 | | 0.2 |
| Cardiovascular disease | 1 | 2.6 | 0 | 1.2 |
| Pnemonia/influenza | | 0.1 | | 0.0 |
| Pulmonary emphysema/asthma | | 0.1 | | 0.0 |
| Cirrhosis of liver | 1 | 0.3 | 0 | 0.1 |
| External causes | | 2.3 | 0 | 0.5 |
| All other | | 1.0 | | 0.3 |

SOURCE: Ott et al., 1976.

(i.e., asbestos, cigarette smoke) or with cancers rarely occurring naturally. Ott et al. (1976) mentioned that the cohort exposed to vinylidene chloride was also exposed to copolymers other than vinyl chloride, and that individuals in the matched control population might have been exposed to a number of other chemicals. However, based on power considerations alone, the Ott et al. study is inadequate for assessing a cancer risk, and cannot be said to support a lesser susceptibility to potential vinylidene chloride carcinogenesis in man.

### 10.5.3 Risk Estimates from Animal Data

The evidence for the carcinogenicity of vinylidene chloride reviewed in this chapter consists primarily of kidney adenocarcinomas diagnosed in treated male Swiss mice. Also, an increase of mammary carcinomas was observed in the female mice and an increase in pulmonary adenomas occurred in both sexes of these mice. An increase in mammary tumors also has been observed in female Sprague-Dawley rats, but was not considered by the authors to be treatment-related (see earlier discussion of animal studies, this chapter). Vinylidene chloride has been shown to act as a tumor initiator in mouse skin, to be mutagenic in several non-mammalian test systems, and to be metabolized to intermediates capable of reacting with cellular macromolecules, including DNA. The overall qualitative weight of evidence for the carcinogenicity of vinylidene chloride in animals is only limited; however, the kidney adenocarcinomas in male Swiss mice (Maltoni et al., 1985) provide data which may be used to estimate an upper limit of incremental risk under the presumption that vinylidene chloride is a potential human carcinogen.

It is important to note that the calculations in this document for estimating in quantitative terms the impact of vinylidene chloride as a carcinogen are made independently of the overall weight of evidence for vinylidene chloride's carcinogenic potential. This is done to answer the question of how large the incremental cancer risk would be if vinylidene chloride were a human carcinogen. However, there is only limited animal evidence for vinylidene chloride carcinogenicity, and the calculations are made only under the supposition that vinylidene chloride is carcinogenic in humans.

10.5.3.1 SELECTION OF ANIMAL DATA SET -- For some chemicals, several studies in different animal species, strains, and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of these data sets to use in the mathematical extrapolation model. A general approach is to use the most sensitive responder, i.e., the data set that gives the highest estimate of lifetime cancer risk, on the assumption that humans are as sensitive as the most sensitive animal species tested. For vinylidene chloride, the Maltoni et al. (1985) inhalation study in Swiss mice is the only animal study in which a statistically significant treatment-related increase in tumors was observed. Swiss mice of both sexes were exposed to 10 and 25 ppm vinylidene chloride 4 hours/day, 4 to 5 days/week for 52 weeks of a total of 121 of weeks. The significant increase of kidney adenocarcinomas in the male mice was the data selected for extrapolating to an upper limit of potential human risk. These tumors were found at the 25 ppm level (25.5% incidence) but not at the 10 ppm level (Table 10-47).

TABLE 10-47. DATA FROM THE MALTONI ET AL. INHALATION STUDY ON MALE MICE

| Animal exposure (ppm daily for 12 months) | Animal lifetime continuous exposure (ppm) | Human lifetime continuous equivalent dose[c] (mg/kg/day) | Number of animals at start | Kidney adenocarcinomas/ number surviving 55 weeks from start |
|---|---|---|---|---|
| 0 | 0 | 0 | 100 | 0/56[a] |
| 0 | 0 | 0 | 90 | 0/70[a] |
| 10 | 0.46 | 0.078 | 30 | 0/25 |
| 25 | 1.15 | 0.195 | 30 | 3/21 (14.3%)[b] |
| 25 | 1.15 | 0.195 | 120 | 25/98(25.5%)[b] |

[a] Groups were combined since responses were not statistically different.

[b] These groups were combined, since responses using the same dose level were not statistically different.

[c] This dose information is derived as shown in Section 10.5.3.2.2.

### 10.5.3.2 INTERSPECIES DOSE CONVERSION

10.5.3.2.1 General Considerations -- A broad classification of the main components to be considered in an extrapolation base assessment, i.e., the bases for extrapolation of dose-carcinogenic response relationships of laboratory animals to man, are: 1) toxicologic data, 2) pharmacokinetics and metabolism, and 3) covalent binding. These components have been outlined and discussed previously by Davidson (1984) and Parker and Davidson (1984).

10.5.3.2.1.1 Toxicologic Data. The toxicity of vinylidene chloride appears to vary with the age, sex, and species of animal exposed (see sections on acute and chronic toxicity). A considerable difference in toxicity also exists between continuous exposure and daily interval exposure. However, there is evidence to suggest (with vinylidene chloride inhalation exposure) a correlation of toxicity to metabolism, with cellular damage occurring after formation of reactive metabolites, including the putative epoxide. This relationship is amply supported by studies demonstrating modulation of toxicity by inhibitors or inducers of $P_{450}$ metabolism.

The cellular damage and toxicity of vinylidene chloride from metabolites formed during inhalation exposure is dependent upon two principal parameters: 1) inhalation concentration (below saturation of metabolic capacity) and 2) duration (length) of exposure. Acute toxicity data are less relevant to the circumstances of the carcinogenicity assays, but chronic and subchronic studies provide directly related data for inhalation toxicity occurring at levels well below saturation of metabolism and relevant to the exposure concentrations used in the Maltoni et al. (1985) carcinogenesis study.

10.5.3.2.1.2 Metabolism. Vinylidene chloride is believed to be metabolized by the microsomal $P_{450}$ monooxygenase system, with the formation of a reactive intermediate epoxide and possibly other reactive metabolites (see

Section 10.1.2, Metabolism). In vivo covalent binding of vinylidene chloride to cellular macromolecules, including DNA, has been demonstrated in the mouse and rat, with binding twofold to threefold greater in kidney than in liver (McKenna et al., 1977; Reitz et al., 1980). The binding is considered to be due to reactive metabolites.

There is evidence that macromolecular covalent binding of metabolites leading to cellular damage is related to the carcinogenic potential of vinylidene chloride, although the primary genotoxic and epigenetic mechanisms resulting in impairment have not yet been satisfactorily established. There is no evidence to show that the metabolic pathways for vinylidene chloride are significantly different, qualitatively or quantitatively, in the mouse, rat, or man.

Experimental studies of the metabolism and pharmacokinetics of vinylidene chloride in man have not been made, but these factors have been relatively extensively studied in two species, the mouse and the rat (McKenna et al., 1977; 1978a,b; Dallas et al., 1983; Putcha et al., 1984; Reitz et al., 1980; Anderson et al., 1979a,b; Filser and Bolt, 1979). Vinylidene chloride in air rapidly equilibrates across the lungs to provide steady-state equilibrium in the rat within 30 to 45 minutes (Dallas et al., 1983; Putcha et al., 1984). At equilibrium the blood concentration (and, hence, the amount of vinylidene chloride in the body) is directly proportional to the inspired air concentration (Dallas et al., 1983). For low inhaled concentrations (0 to 150 ppm), vinylidene chloride is metabolized at a constant rate consistent with a constant blood concentration presented to the liver, the principal site of metabolism, by the equilibrium maintained by pulmonary uptake (Dallas et al., 1983; Putcha et al., 1984; Anderson et al., 1979a,b; Filser and Bolt, 1983). Metabolic elimination of vinylidene chloride is saturable and dose-dependent, with saturation of metabolism in rodents occurring at inhalation concentrations of about 150 to 200 ppm or about 50 mg/kg oral ingestion (Dallas et al., 1983; Anderson et al., 1979a,b; Filser and Bolt, 1979). Below saturation concentrations, metabolism is essentially first-order and virtually 100% (Dallas et al., 1983; Filser and Bolt, 1979). The processes of oral absorption and metabolism of vinylidene chloride are both virtually complete at least up to 50 mg/kg in both rats and mice (McKenna et al., 1978b; Putcha et al., 1984).

10.5.3.2.1.3 Covalent Binding, DNA Alkylation. Covalent binding of vinylidene chloride closely correlates with metabolite formation. McKenna et al. observed that covalently bound $^{14}C$ activity in tissues was three times greater in the kidney than in the liver, and was five times greater for mouse than rat on a direct body weight (mg/kg) basis.

Reitz et al. (1980) found minimal DNA alkylation in liver and kidney of both mouse and rat exposed to 10 and 50 ppm vinylidene chloride by inhalation for 6 hours. The alkylation that occurs is proportional to metabolite formation. The kidney shows a twofold greater alkylation (rat) and tenfold greater alkylation (mouse) than the liver.

10.5.3.2.2 Calculation of Human Equivalent Doses from Animal Data -- The usual approach for making interspecies comparisons has been to use standardized scaling factors. The allometric equation:

$$\text{Dose (for a given tumor incidence)} = aW^n$$

where W = body weight, is appropriate to use for interspecies extrapolation of carcinogenicity bioassay data from laboratory animals to man. In extrapolating dosage from mouse or rat to man, it has long been customary to use either body weight (n = 1) or surface area (n = 0.67) as the main criterion.

Although reliable interspecies information on the relationship of dosage to carcinogenesis is not available, in general other toxicologic, metabolic, and pharmacokinetic data correlate with interspecies body weight with the power of 0.67 to 0.75 for the majority of compounds studied. Thus, in the absence of comparative toxicologic, metabolic, and pharmacokinetic data for a given suspect carcinogen, the extrapolation, on the basis of surface area ($W^{0.67}$), is considered to provide the best dose extrapolation prediction, with a greater probability of correctness. When adequate toxicologic, metabolic, and pharmacokinetic data are available for a suspect carcinogen in two or more species of laboratory animals or man, the experimental data should be used to determine and to justify the extrapolation base. In view of the finding that vinylidene chloride metabolism, and hence its potential for cellular damage, in mouse and rat correlates with body surface area, as shown below, using surface area for the extrapolation of a vinylidene chloride human equivalent dose would seem more reasonable, especially because it correlates better with the known parameters of metabolism and physiology in general.

The actual experimental observations allow a calculation of assimilated dose and metabolite formation for the daily vinylidene chloride inhalation exposure experienced by the Swiss mice of the Maltoni et al. (1985) carcinogenicity bioassay from the McKenna et al. (1977) balance study. McKenna et al. exposed male mice and rats to $^{14}C$-vinylidene chloride by inhalation (10 ppm for 6 hours), and estimated the body burden from radioactivity recovered in expired air, urine, feces, and carcass. At this exposure level virtually all of the assimilated dose was metabolized in both species; the body burdens and amounts metabolized were as follows:

|  | mg/kg | mg/animal |
|---|---|---|
| Mouse (35 g) | 5.30 | 0.186 |
| Rat (250 g) | 2.89 | 0.723 |

In the carcinogenicity bioassay of Maltoni et al. (1985) the mice were exposed 4 hours/day at 10 to 25 ppm. At these low inhalation concentrations, plateau or steady-state blood concentrations of vinylidene chloride are quickly reached, and the rates of metabolism are constant with time and are proportional to the inhalation concentration (Dallas et al., 1983). Therefore, the body burden (and amount metabolized) can be extrapolated from McKenna's 6-hour exposure to Maltoni's 4-hour exposures by a simple proportional adjustment of 4/6. Extrapolation from 10 ppm to 25 ppm can be made similarly, to yield the following metabolic loads for Maltoni's animals:

|  | 10 ppm |  | 25 ppm |  |
|---|---|---|---|---|
|  | mg/kg | mg/animal | mg/kg | mg/animal |
| Mouse (35 g) | 3.53 | 0.124 | 8.83 | 0.309 |
| Rat (250 g) | 1.93 | 0.483 | 4.82 | 1.205 |
| Ratio (rat/mouse) | 0.55 | 3.89 | 0.55 | 3.89 |

The ratio of the amount metabolized in the rat to the amount metabolized in the mouse equals 3.89. Thus, the metabolism and pharmacokinetics for mice and rats can be considered proportional on a $W^{0.67}$ "surface area" allometric base (as $[250/35]^{0.67} = 3.71$) but not on a $W^{1.0}$ "mg/kg" base. Therefore, based on this information, the most appropriate approach to extrapolating mouse carcinogenicity data to man is to scale the total assimilated dosage on a $W^{0.67}$ base with a scaling factor for mouse to man of $(70/0.035)^{0.67} = 159$. The lifetime average daily exposure (LAE) for the Maltoni mice is given by:

$$LAE_M = \frac{52 \text{ weeks}}{121 \text{ weeks}} \times \frac{4.5 \text{ days}}{7 \text{ days}} \times \text{body burden, mg/day}$$

and for humans,

$$LAE_H = LAE_M \times 159$$

On this basis, the lifetime average human equivalent doses for the vinylidene chloride mouse carcinogenicity bioassay, expressed in units of mg/day, mg/$m^2$ surface area/day, and mg/kg/day, are as follows:

| Inhalation concentration (ppm) | Equivalent lifetime average human dose[a] | | |
|---|---|---|---|
| | mg/day | (mg/$m^2$)/day | (mg/kg)/day |
| 10 | 5.44 | 2.94 | 0.078 |
| 25 | 13.60 | 7.35 | 0.195 |

[a] Where the average weight of the male mice is assumed as 0.035 kg and that of the standard man as 70 kg with 1.85 $m^2$ surface area (Diem and Lentner, 1970).

### 10.5.3.3 CHOICE OF RISK MODEL

10.5.3.3.1 General Considerations -- The data used for quantitative estimation of unit risk for vinylidene chloride were taken from a lifetime animal study. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses at incidence determined by the extrapolation model. However, there is no solidly accepted scientific basis for any mathematical extrapolation model that relates chemical carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons, such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. It is necessary, therefore, to depend on our current knowledge of the mechanisms of carcinogenesis for guidance as to the correct risk model to use.

At the present time, the dominant view is that most cancer-causing agents also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal type of biological response, which is characteristic of mutagenesis, is associated with low-dose linearity and a nonthreshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. At higher doses, there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The low-dose linearity and nonthreshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxins in the diet). There is also some evidence from animal experiments that is consistent with the linear nonthreshold model (e.g., the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Based on the above evidence of low-dose linearity, and because very few compounds exhibit low-dose responses that are supralinear, the nonthreshold model, which is linear at low doses, has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper limit for the risk, i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

The methods used by the CAG for quantitative assessment are consistently conservative, i.e., tending toward high estimates of risk. The most important part of the methodology contributing to this conservatism is the linear nonthreshold extrapolation model. There are a variety of other extrapolation models that could be used, all of which would give lower risk estimates. These alternative models have not been used by the CAG in the following analysis because it feels that the limited evidence of carcinogenicity for vinylidene chloride does not warrant that complete an analysis. Kidney adenocarcinomas in male mice occur at only one dose level, thus allowing an estimation of only one parameter. Furthermore, the CAG feels that with the limited data available from the animal bioassays, very little is known about the true shape of the dose-response curve at low environmental levels. The position is taken by the CAG that the risk estimates obtained by use of the low-dose linear nonthreshold model are plausible upper limits, and that the true risk could be lower.

Extrapolations from animals to humans could also be done on the basis of relative weights or measures other than relative surface area. The general approach is to use the extrapolation base (mg/kg, surface area, etc.) that can be appropriately justified by the experimental data from animals and man, although it is usually not clear which extrapolation base is the most appropriate for the carcinogenic response per se. In the case where there is insufficient data or an absence of experimental data to determine an extrapolation base either directly or indirectly, the most generally employed and conservative method is used, i.e., extrapolation from animal dose to a human equivalent dose on the basis of relative surface area ($W^{2/3}$). For the vinylidene chloride inhalation studies in mice (Maltoni et al., 1985) the use of an extrapolation based on surface area ($W^{2/3}$) rather than directly on body weight ($W^{1.0}$), results in a higher unit risk estimate than direct body weight extrapolation, or assuming that a certain concentration in ppm or $\mu g/m^3$ in the experimental animals is equivalent to the same concentration in humans. However, for vinylidene chloride, experimental data on metabolism and kinetics are available and can be used in dose extrapolation from mouse to man.

10.5.3.3.2 Mathematical Description of the Low-Dose Extrapolation Model -- Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \ i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\ [-(q_1 d + q_2 d^2 + \ldots + q_k d^k)]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d or the effect of treatment.

The point estimate of the coefficents $q_i$, $i = 0, 1, 2, \ldots, k$, and consequently the extra risk function $P_t(d)$ at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL83, developed by Howe (1983). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, $q_1^*$, on parameter $q_1$. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk and $R/q_1^*$ is a 95% lower confidence limit on the dose producing an extra risk of R. Let $L_0$ be the maximum value of the log-likelihood function. The upper limit, $q_1^*$, is calculated by increasing $q_1$ to a value $q_1^*$ such that when the loglikelihood is remaximized subject to this fixed value, $q_1^*$, for the linear coefficient, the resulting maximum value of the log-likelihood $L_1$ satisfies the equation

$$2\ (L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper-limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope, $q_1^*$, is taken as an upper-bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.)

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment including the control group. A positive response to vinylidene chloride exposure occurs at only one dose level in the Maltoni study. Therefore, only one term in the polynomial, other than the constant, can be estimated.

### 10.5.3.4 CALCULATION OF UNIT RISK

10.5.3.4.1 Definition of Unit Risk -- This section deals with the unit risk for vinylidene chloride in air and water and the potency of vinylidene chloride relative to other carcinogens that the CAG has evaluated. The unit risk estimate for an air or water pollutant is defined as the increased lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 $\mu g/m^3$ of the agent in the air they breathe, or to 1 $\mu g/L$ in the water they drink. This calculation is done to estimate in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to give a crude indication of the population risk which might be associated with air or water exposure to these agents, if the actual exposures are known.

For most cases of interest to risk assessment, the 95% upper-limit risk associated with dose units/day, as obtained by means of the GLOBAL83 computer program (Howe, 1983), can be adequately approximated by $P(d) = 1 \exp-(q_1^{*}d)$. A "unit risk" in units X is the risk corresponding to an exposure of X = 1. To estimate this value we simply find the number of dose units/day that correspond to one unit of X, and substitute this value into the above relationship.

10.5.3.4.2 Interpretation of Unit Risk Estimates -- For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. There may be important differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. In addition, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative carcinogenic potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure, although ordinarily the risk should be independent of route of exposure except in special circumstances such as nasal or lung carcinoma with inhalation exposure, or forestomach tumors with gavage administration.

The quantitative aspect of carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk, i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be lower. The risk estimates for vinylidene chloride presented in this document should not be regarded as immutable representations of the true cancer risks; they are calculated only under the presumption that vinylidene chloride is carcinogenic. However, the estimates presented may be factored into regulatory decisions to the extent that the concept of upper risk limits for vinylidene chloride are found to be useful.

This chapter gives the estimates of $q_1^*$, 95% upper-limit slope from the linearized multistage model.

#### 10.5.3.4.3 Estimation of Unit Risk Using the Maltoni et al. (1985) Inhalation Study --

10.5.3.4.3.1 Unit Risk for Air. The only animal study showing a significant increase in tumors is the inhalation study of Maltoni et al. (1985), in which male Swiss mice developed kidney adenocarcinomas. The pertinent long-term (52 weeks) exposure-response data from this study are presented in Table 10-47. The two control groups were combined, as well as the two groups treated at 25 ppm, since the responses within treatment groups were not statistically different. Lacking information on early mortality and scheduled sacrifice, the number surviving to the time of the first kidney adenocarcinoma is used as the denominator.

The calculation of the cancer risk estimates by the multistage model, allowing for a two-stage process, yields a maximum likelihood estimate (MLE) for the linear term of $q_1 = 0$, and a quadratic term of $q_2 = 6.79\ (\text{mg/kg/day})^{-2}$.

The 95% upper limit on the linear term is

$$q_1^* = 1.16\ (\text{mg/kg/day})^{-1}$$

The unit risk, $q_1^*$, can also be expressed in terms of excess cancer risk per ppm of continuous lifetime exposure to vinylidene chloride. To express the unit risk in terms of ppm, the animal data are used to calculate a human equivalency (see Section 10.5.3.2.2) in mg/day or mg/kg/day. A continuous lifetime exposure to vinylidene chloride in ppm calculated from the experimental exposure is:

$$10\ \text{ppm} \times 4/24 \times 4.5/7 \times 52/121 = 0.46\ \text{ppm}$$

Thus, if a 0.46 ppm continuous lifetime exposure results in a daily human dose of 0.078 mg/kg/day, then 1 ppm continuous lifetime exposure results in a daily human dose of 0.17 mg/kg. The conversion follows simply as:

$$q_1^* = 1.16\ (\text{mg/kg/day})^{-1} \times \frac{0.17\ \text{mg/kg/day}}{\text{ppm}} = 2.0 \times 10^{-1}\ (\text{ppm})^{-1}$$

or

$$q_1^* = 2.0 \times 10^{-4}\ (\text{ppb})^{-1}$$

The same value of $q_1^*$ in units of $(\text{ppm})^{-1}$ or $(\text{ppb})^{-1}$ could have been derived directly by extrapolating on a direct ppm animal-to-human equivalence. This is because of the proportionality assumptions necessary to determine equivalent doses in Table 10-47. To express the unit risk in terms of $\mu g/m^3$, the following conversion is used:

$$1\ \mu g/m^3 = 1\ (\mu g/m^3) \times 10^{-3}\ (m^3/L)/1.2\ (g/L) \times MW\ (VDC)/MW\ (air) =$$
$$10^{-3}/(1.2 \times 97/28.8)\ \mu g/g = 2.5 \times 10^{-4}\ ppm$$

For a lifetime continuous exposure to 1 $\mu g/m^3$ of vinylidene chloride, the corresponding estimate is

$$q_1^* = 2.0 \times 10^{-1}\ (ppm)^{-1} \times 2.5 \times 10^{-4} = 5.0 \times 10^{-5}\ (\mu g/m^3)^{-1}$$

10.5.3.4.3.2 Unit Risk for Drinking Water. The unit risk for water ingestion can be derived using two basic approaches: a) converting the doses in the Maltoni et al. (1985) inhalation study to an equivalent oral dose and using the kidney tumor incidence data, and b) estimating an upper-limit value from the negative data of the Quast et al. (1983) drinking water study in Sprague-Dawley rats or from the negative NCI/NTP (1982) gavage study in Fischer 344 rats and B6C3F1 mice.

Both approaches have limitations. Although the first approach is based on positive tumor response data, the uncertainty of determining the effective uptake via inhalation is a limitation. The data of McKenna et al. (1977) help to clarify the situation in rats, but the lack of human uptake data via the inhalation route is still a limitation. The second approach assumes that a response occurs via ingestion, although there is no direct evidence that this is the case.

For the first approach, extrapolation from the Maltoni et al. inhalation study above yields a 95% upper limit on the linear term of $q_1^* = 1.16$ (mg/kg/

day)$^{-1}$. The upper-limit incremental unit risk for drinking water, assuming 2 L/day of intake and 100% absorption, can be estimated by first noting that the intake from a concentration of 1 μg/L is

$$1\ \mu g/L \times 2\ L/day \times 10^{-3}\ mg/\mu g \times 1/70\ kg = 2.86 \times 10^{-5}\ mg/kg/day$$

The risk from this intake is

$$\begin{aligned} R &= 1.16 \times 2.86 \times 10^{-5} \\ &= 3.3 \times 10^{-5} \end{aligned}$$

which is the unit risk for 1 μg/L in drinking water derived from the Maltoni et al. inhalation data.

In the second approach, the data on total tumors in the Quast et al. (1983) study (Table 10-40) in male rats is used. After converting the average number of tumors per animal to expected incidence of animals with tumors, the doses were converted to their human equivalents using the surface area factor, and the linearized multistage model was used to find the upper 95% confidence limit of the slope, $q_1^*$. The result is $q_1^* = 0.159$ (mg/kg/day)$^{-1}$.

The same method was used for the data of the NCI/NTP study in rats and mice (Table 10-43). In this calculation, data on the male rat adrenal pheochromocytomas, the male rat subcutaneous fibromas, and the female mouse lymphomas were used. The upper-bound slope estimates were as follows:

| Data set | Upper-bound slope estimate $(mg/kg/day)^{-1}$ |
|---|---|
| Male rat adrenal | 0.577 |
| Male rat fibromas | 0.284 |
| Female mouse lymphomas | 0.364 |

The range of these upper-bound estimates from the negative studies is from 0.16 to 0.58 $(mg/kg/day)^{-1}$. The interpretation of this range is that if a response does occur via ingestion of vinylidene chloride, the slope is not higher than 0.58 $(mg/kg/day)^{-1}$. Assuming 2 L/day of drinking water intake, the unit risk from a water concentration of 1 μg/L can be calculated as follows: the dose from a concentraton of 1 μg/L as shown above is $2.86 \times 10^{-5}$ mg/kg/day. The upper-bound incremental risk resulting from the dose is

$$R = 0.58 \ (mg/kg/day)^{-1} \times 2.86 \times 10^{-5} \ mg/kg/day$$
$$= 1.66 \times 10^{-5}$$

This is the upper-bound unit risk estimate for 1 μg/L of vinylidene chloride in drinking water. This value is approximately one-half of the estimate obtained directly from the positive Maltoni et al. inhalation data. The estimate based on oral studies is chosen over the one derived from the Maltoni at al. inhalation study because the oral route of administration is more appropriate.

### 10.5.4 Risk Estimates from Epidemiologic Data

10.5.4.1 SELECTION OF EPIDEMIOLOGIC DATA SETS -- Whenever possible, human data are used in preference to animal bioassay data. If epidemiologic studies and sufficiently valid exposure information are available for a compound, they may be used in several ways. If these data show a carcinogenic effect, they are analyzed to give an estimate of the linear dependence of cancer rates on lifetime average dose, which is equivalent to the factor $B_H$ (see Section 10.5.4.2 for definition). If no carcinogenic effects are seen in the epidemiologic studies when positive animal evidence is available, the assumption is that a risk does exist, but that it is too small to be observable in the epidemiologic data. An upper limit to the cancer incidence is then calculated, assuming hypothetically that the true incidence is below the level of detection in a human cohort of the size studied.

Very little information exists that can be used for extrapolating from high-exposure occupational studies to low environmental levels. However, if a number of simplifying assumptions are made, it is possible to construct a crude dose-response model whose parameters can be estimated using vital statistics, epidemiologic studies, and estimates of worker exposures. Thus, the Ott et al. (1976) study provides data which may be used to calculate 95% upper-limit estimates on relative risk, although this study does not provide evidence for the carcinogenicity of vinylidene chloride.

10.5.4.2 DESCRIPTION OF RISK MODEL -- In human studies, the response is measured in terms of the relative risk of the exposed cohort of individuals as compared to the control group. The mathematical model employed assumes that for low exposures the lifetime probability of death from a specific cancer, $P_0$, may be represented by the linear equation

$$P_0 = A + B_H X$$

where A is the lifetime probability in the absence of the agent, and X is the average lifetime exposure to environmental levels in units such as ppm. The factor $B_H$ is the increased probability of cancer associated with each unit increase of the agent in air.

If we make the assumption that R, the relative risk of lung cancer for exposed workers in comparison to the general population, is independent of the length or age of exposure but depends only upon the average lifetime exposure, it follows that

$$R = \frac{P}{P_0} = \frac{A + B\ (X_1 + X_2)}{A + B_H\ (X_1)}$$

or

$$RP_0 = A + B_H\ (X_1 + X_2)$$

where $X_1$ = lifetime average daily exposure to the agent for the general population, $X_2$ = lifetime average daily exposure to the agent in the occupational setting, and $P_0$ = lifetime probability of dying of cancer with no or negligible epichlorohydrin exposure.

Substituting $P_0 = A + B_H X_1$ and rearranging gives

$$B_H = P_0\ (R - 1)/X_2$$

To use this model, estimates of R and $X_2$ must be obtained from the epidemiologic studies. The value $P_0$ is derived by means of life table methodology from the age-cause-specific combined death rates for males found in the U.S. Vital Statistics tables. For total cancer, the estimate of $P_0$ is 0.23. This model is used in section 10.5.3.10, which deals with unit risk estimates based on human studies.

10.5.4.3 CALCULATION OF UPPER LIMITS OF RISK -- Although the epidemiologic studies on vinylidene chloride provide no evidence for its carcinogenicity, they can still be used to provide information on an upper limit of risk to be expected. The CAG adopts this procedure of using negative epidemiologic studies for providing upper limits when animal studies are positive. Such use of negative human studies can modify estimates of potency from the positive animal studies.

10.5.4.4 COMPARING ANIMAL AND HUMAN INHALATION STUDIES -- This section investigates whether or not the estimates of cancer risk extrapolated from kidney adenocarcinoma response in the Maltoni et al. (1985) inhalation study on Swiss male mice reasonably reflect the observed response of the Ott et al. (1976) study of vinylidene chloride-exposed humans. Both animals and humans were exposed to comparable levels but for different portions of their lifetimes--10 ppm and 25 ppm for 4 hours/day, 4-5 days/week for 52 weeks for the animals, and $< 5$ ppm to about 70 ppm for an 8-hour time-weighted average (TWA), 5 days/week for an average exposure of about 5.7 years for humans. Average follow-up for the animals was 121 weeks; for the 138 vinylidene chloride workers average follow-up was about 15.7 years. Based on the figures in Table 10-45, we estimate the average 8-hour TWA exposure to have been 16.5 ppm over the exposure period.

The observed effects in the workers were 5 deaths overall (7.5 expected) and one cancer death (1.1 expected). As discussed in the epidemiology section, disregarding latent period, at least 12 cancer deaths (relative risk of 10.6) would have had to occur to be able to detect a significantly elevated risk at the $p < 0.05$ level with 80% power. In order to determine the probability of human response predicted by animal extrapolation, we must make some assumptions of proportionality which might not hold for vinylidene chloride dose-response. Specifically, we must assume proportional responses from 5.7 years average exposure and 15.7 years average observation (out of 50 years possible remaining for workers). For extrapolation from animal data we use the MLE estimates, which are nonlinear (quadratic) in the experimental range. The MLE extrapolations to humans converted to ppm are $q_0 = 0$ and $q_2 = 0.195\ (\text{ppm})^{-2}$. Based on the above assumptions of proportionality, we can estimate individual cancer risk to the average worker as the lifetime predicted cancers (based on animal risk extrapolation to the human exposure conditions) as follows:

$$P = 1 - \exp\left[- 0.195\ (\text{ppm})^{-2} \left(16.5\ \text{ppm} \times \frac{8}{24} \times \frac{240}{365} \times \frac{5.7}{50}\right)^2\right] = 0.033$$

with an upper 95% confidence limit of

$$1 - \exp\,(- 0.20 \times 16.5 \times 8/24 \times 240/365 \times 5.7/50) = 0.79$$

For 138 employees at risk for an average of 15.7 years, we would expect to see

$$138 \times 0.033 \times \frac{15.7}{50} = 1.44 \text{ cancers.}$$

This figure is not significantly ($p > 0.25$) larger than that observed, based on the Poisson probability test (the probability of observing as few as 1 cancer death if 2.5 [1.44 + 1.1] are expected). The risk based on animal extrapolation appears consistent with the human data.

Even if we consider a 15-year latent period for expression of the cancer effect, the results do not change appreciably. Although the effects for this subcohort cannot be directly predicted, we note that 83 of the 138 had a potential follow-up of at least 15 years and that 103 of the 138 were still working as of the observation cutoff date. Thus, it seems reasonable to assume that at least half of the original cohort would have had sufficient exposure and latent period to have developed an effect. However, the sample size is still very small.

However, the weaknesses of the Ott et al. study become still more apparent when we try to use its dose-response information directly. In order to make an estimate from this negative study, our procedure is to base the estimate on a 95% upper limit. The model, presented in section 10.5.3.6, requires an estimate of the 95% upper limit of the observed SMR of 91 (95% upper confidence limit = 505) and an estimate of continuous lifetime equivalent exposure. As presented above for the cohort of 138, this is

$$16.5 \text{ ppm} \times \frac{8}{24} \times \frac{240}{365} \times \frac{5.7}{50} = 0.41 \text{ ppm}$$

Thus, based on the relative risk model for human data, the 95% upper-limit unit risk, $B^*$, for a continuous lifetime exposure to 1 ppm vinylidene chloride in air is

$$B^* = \frac{P_0(R-1)}{X} = \frac{0.23\ (4.05)}{0.41} = 2.27\ (\text{ppm})^{-1}$$

where $P_0 = 0.23$ is the approximate lifetime probability of death from cancer based on 1976 U.S. Vital Statistics death rates. This is greater than the 95% upper-limit estimate $q_1^* = 0.20\ (\text{ppm})^{-1}$ from the animal study. The basic problem arises from the weakness of the Ott study due to the small sample size and short follow-up. Thus, based on the above analyses, we conclude that the extrapolations from the animal data are consistent with the human response. In addition, because of the uncertainty due to the small size and limited observation in the epidemiology study, the extrapolation from the animal study cannot be dismissed as too high. The 95% upper-limit incremental unit risk from the animal study, $q_1^* = 0.20\ (\text{ppm})^{-1}$ is chosen as the 95% upper-limit incremental unit risk estimate, because it is lower than the 95% upper-limit estimate, $q_1^* = 2.27\ (\text{ppm})^{-1}$ based on the negative human study.

### 10.5.5 Relative Carcinogenic Potency

10.5.5.1 DERIVATION -- One of the uses of the concept of unit risk is to compare the relative potency of carcinogens. To estimate the relative potency on a per mole basis, the unit risk slope factor is multiplied by the molecular weight, and the resulting number is expressed in terms of $(\text{mmol/kg/day})^{-1}$. This is called the relative potency index.

Figure 10-7 is a histogram representing the frequency distribution of potency indices of 54 chemicals evaluated by the CAG as suspect carcinogens. The actual data summarized by the histogram are presented in Table 10-48. Where human data were available for a compound, they were used to calculate the index. Where no human data were available, animal oral studies and animal inhalation studies were used, in that order. Animal oral studies were selected over animal inhalation studies because most of the chemicals have been tested with animal oral studies, thus better allowing potency comparisons by route.

10.5.5.2 POTENCY INDEX -- The potency index for vinylidene chloride based on kidney adenocarcinomas in the Maltoni et al. (1985) inhalation bioassy is $1.1 \times 10^{+2}\ (\text{mmol/kg/day})^{-1}$. This is derived as follows: the 95% upper-limit slope estimate from the Maltoni study is $1.16\ (\text{mg/kg/day})^{-1}$. Multiplying by

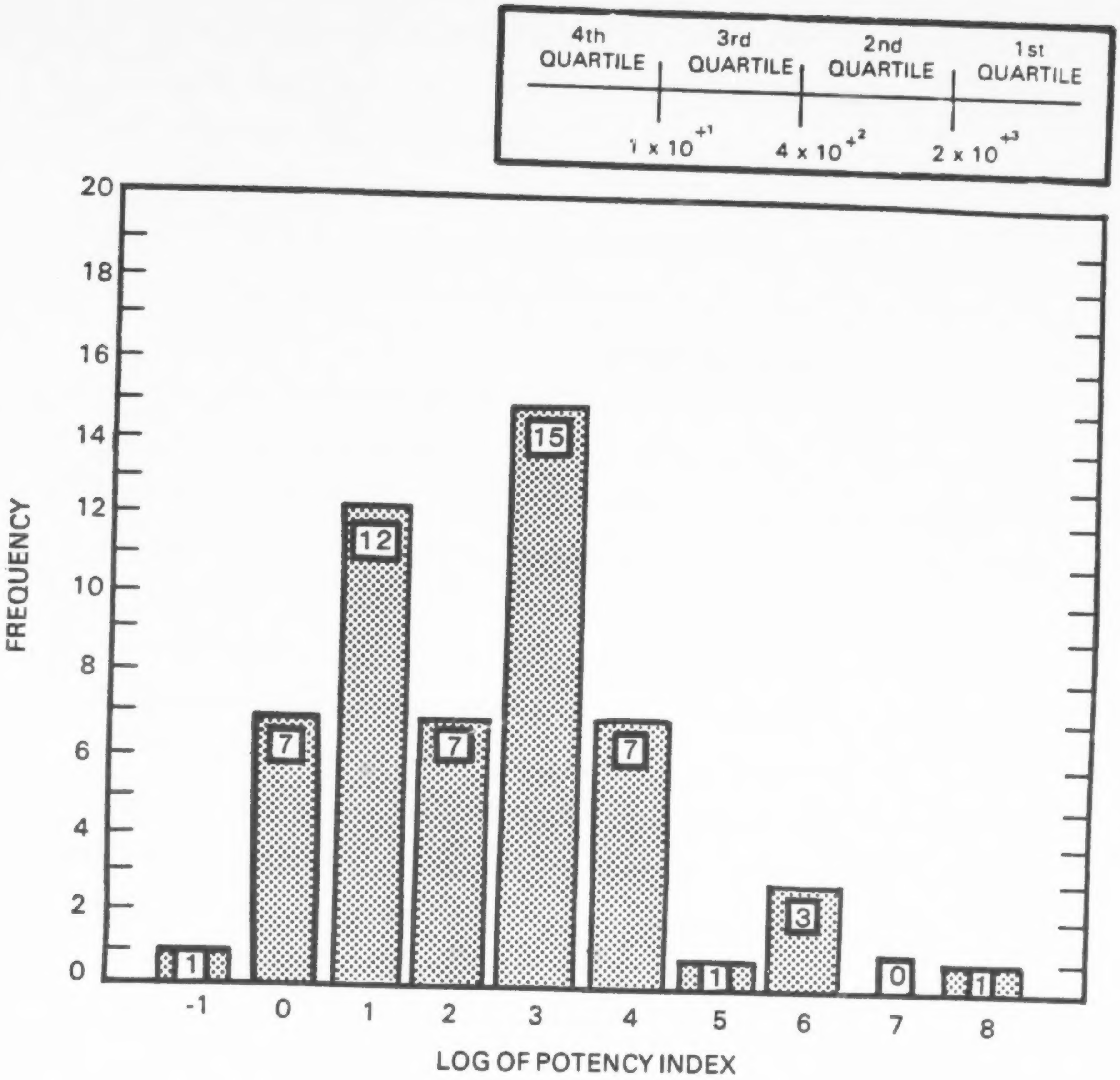

Figure 10-7. Histogram representing frequency distribution of the potency indices of 54 suspect carcinogens evaluated by the Carcinogen Assessment Group.

## TABLE 10-48. RELATIVE CARCINOGENIC POTENCIES AMONG 54 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS

| Compounds | CAS Number | Level of evidence[a] | | Grouping based on IARC criteria | Slope[b] $(mg/kg/day)^{-1}$ | Molecular weight | Potency index[c] | Order of magnitude ($\log_{10}$ index) |
|---|---|---|---|---|---|---|---|---|
| | | Humans | Animals | | | | | |
| Acrylonitrile | 107-13-1 | L | S | 2A | 0.24(W) | 53.1 | $1x10^{+1}$ | +1 |
| Aflatoxin $B_1$ | 1162-65-8 | L | S | 2A | 2900 | 312.3 | $9x10^{+5}$ | +6 |
| Aldrin | 309-00-2 | I | L | 2B | 11.4 | 369.4 | $4x10^{+3}$ | +4 |
| Allyl chloride | 107-05-1 | | | | $1.19x10^{-2}$ | 76.5 | $9x10^{-1}$ | 0 |
| Arsenic | 7440-38-2 | S | I | 1 | 15(H) | 149.8 | $2x10^{+3}$ | +3 |
| B[a]P | 50-32-8 | I | S | 2B | 11.5 | 252.3 | $3x10^{+3}$ | +3 |
| Benzene | 71-43-2 | S | S | 1 | $2.9x10^{-2}$(W) | 78 | $2x10^{0}$ | 0 |
| Benzidene | 92-87-5 | S | S | 1 | 234(W) | 184.2 | $4x10^{+4}$ | +5 |
| Beryllium | 7440-41-7 | L | S | 2A | 2.6 | 9 | $2x10^{+1}$ | +1 |
| 1,3-Butadiene | 106-99-0 | I | S | 2B | $1.0x10^{-1}$(I) | 54.1 | $5x10^{0}$ | +1 |
| Cadmium | 7440-43-9 | L | S | 2A | 6.1(W) | 112.4 | $7x10^{+2}$ | +3 |
| Carbon tetrachloride | 56-23-5 | I | S | 2B | $1.30x10^{-1}$ | 153.8 | $2x10^{+1}$ | +1 |
| Chlordane | 57-74-9 | I | L | 3 | 1.61 | 409.8 | $7x10^{+2}$ | +3 |

(continued on the following page)

TABLE 10-48. (continued)

| Compounds | CAS Number | Level of evidence[a] Humans | Level of evidence[a] Animals | Grouping based on IARC criteria | Slope[b] $(mg/kg/day)^{-1}$ | Molecular weight | Potency index[c] | Order of magnitude ($\log_{10}$ index) |
|---|---|---|---|---|---|---|---|---|
| Chlorinated ethanes | | | | | | | | |
| 1,2-Dichloroethane | 107-06-2 | I | S | 2B | $9.1x10^{-2}$ | 98.9 | $9x10^{0}$ | +1 |
| hexachloroethane | 67-72-1 | I | L | 3 | $1.42x10^{-2}$ | 236.7 | $3x10^{0}$ | 0 |
| 1,1,2,2-Tetrachloroethane | 79-34-5 | I | L | 3 | 0.20 | 167.9 | $3x10^{+1}$ | +1 |
| 1,1,2-Trichloroethane | 79-00-5 | I | L | 3 | $5.73x10^{-2}$ | 133.4 | $8x10^{0}$ | +1 |
| Chloroform | 67-66-3 | I | S | 2B | $7x10^{-2}$ | 119.4 | $8x10^{0}$ | +1 |
| Chromium VI | 7440-47-3 | S | S | 1 | 41(W) | 100 | $4x10^{+3}$ | +4 |
| DDT | 50-29-3 | I | S | 2B | 0.34 | 354.5 | $1x10^{+2}$ | +2 |
| Dichlorobenzidine | 91-94-1 | I | S | 2B | 1.69 | 253.1 | $4x10^{+2}$ | +3 |
| 1,1-Dichloroethylene (Vinylidene chloride) | 75-35-4 | I | L | 3 | 1.16(I) | 97 | $1x10^{+2}$ | +2 |
| Dichloromethane (Methylene chloride) | 75-09-2 | I | L | 3 | $6.3x10^{-4}$(I) | 84.9 | $5x10^{-2}$ | -1 |
| Dieldrin | 60-57-1 | I | S | 2B | 30.4 | 380.9 | $1x10^{+4}$ | +4 |
| 2,4-Dinitrotoluene | 121-14-2 | I | S | 2B | 0.31 | 182 | $6x10^{+1}$ | +2 |
| Diphenylhydrazine | 122-66-7 | I | S | 2B | 0.77 | 180 | $1x10^{+2}$ | +2 |
| Epichlorohydrin | 106-89-8 | I | S | 2B | $9.9x10^{-3}$ | 92.5 | $9x10^{-1}$ | 0 |
| Bis(2-chloroethyl)ether | 111-44-4 | I | S | 2B | 1.14 | 143 | $2x10^{+2}$ | +2 |

(continued on the following page)

TABLE 10-48. (continued)

| Compounds | CAS Number | Level of evidence[a] Humans | Level of evidence[a] Animals | Grouping based on IARC criteria | Slope[b] (mg/kg/day)$^{-1}$ | Molecular weight | Potency index[c] | Order of magnitude ($\log_{10}$ index) |
|---|---|---|---|---|---|---|---|---|
| Bis(chloromethyl)ether | 542-88-1 | S | S | 1 | 9300(I) | 115 | 1x10$^{+6}$ | +6 |
| Ethylene dibromide (EDB) | 106-93-4 | I | S | 2B | 41 | 187.9 | 8x10$^{+3}$ | +4 |
| Ethylene oxide | 75-21-8 | L | S | 2A | 3.5x10$^{-1}$(I) | 44.1 | 2x10$^{+1}$ | +1 |
| Heptachlor | 76-44-8 | I | S | 2B | 3.37 | 373.3 | 1x10$^{+3}$ | +3 |
| Hexachlorobenzene | 118-74-1 | I | S | 2B | 1.67 | 284.4 | 5x10$^{+2}$ | +3 |
| Hexachlorobutadiene | 87-68-3 | I | L | 3 | 7.75x10$^{-2}$ | 261 | 2x10$^{+1}$ | +1 |
| Hexachlorocyclohexane | | | | | | | | |
| technical grade | | | | | 4.75 | 290.9 | 1x10$^{+3}$ | +3 |
| alpha isomer | 319-84-6 | I | S | 2B | 11.12 | 290.9 | 3x10$^{+3}$ | +3 |
| beta isomer | 319-85-7 | I | L | 3 | 1.84 | 290.9 | 5x10$^{+2}$ | +3 |
| gamma isomer | 58-89-9 | I | L | 2B | 1.33 | 290.9 | 4x10$^{+2}$ | +3 |
| Hexachlorodibenzodioxin | 34465-46-8 | I | S | 2B | 6.2x10$^{+3}$ | 391 | 2x10$^{+6}$ | +6 |
| Nickel | 7440-02-0 | L | S | 2A | 1.15(W) | 58.7 | 7x10$^{+1}$ | +2 |
| Nitrosamines | | | | | | | | |
| Dimethylnitrosamine | 62-75-9 | I | S | 2B | 25.9(not by $q_1^*$) | 74.1 | 2x10$^{+3}$ | +3 |
| Diethylnitrosamine | 55-18-5 | I | S | 2B | 43.5(not by $q_1^*$) | 102.1 | 4x10$^{+3}$ | +4 |
| Dibutylnitrosamine | 924-16-3 | I | S | 2B | 5.43 | 158.2 | 9x10$^{+2}$ | +3 |
| N-nitrosopyrrolidine | 930-55-2 | I | S | 2B | 2.13 | 100.2 | 2x10$^{+2}$ | +2 |
| N-nitroso-N-ethylurea | 759-73-9 | I | S | 2B | 32.9 | 117.1 | 4x10$^{+3}$ | +4 |

(continued on the following page)

TABLE 10-48. (continued)

| Compounds | CAS Number | Level of evidence[a] |  | Grouping based on IARC criteria | Slope[b] (mg/kg/day)$^{-1}$ | Molecular weight | Potency index[c] | Order of magnitude (log$_{10}$ index) |
|---|---|---|---|---|---|---|---|---|
|  |  | Humans | Animals |  |  |  |  |  |
| N-nitroso-N-methylurea | 684-93-5 | I | S | 2B | 302.6 | 103.1 | 3x10$^{+4}$ | +4 |
| N-nitroso-diphenylamine | 86-30-6 | I | S | 2B | 4.92x10$^{-3}$ | 198 | 1x10$^{0}$ | 0 |
| PCBs | 1336-36-3 | I | S | 2B | 4.34 | 324 | 1x10$^{+3}$ | +3 |
| Phenols |  |  |  |  |  |  |  |  |
| 2,4,6-Trichlorophenol | 88-06-2 | I | S | 2B | 1.99x10$^{-2}$ | 197.4 | 4x10$^{0}$ | +1 |
| Tetrachlorodibenzo-p-dioxin (TCDD) | 1746-01-6 | I | S | 2B | 1.56x10$^{+5}$ | 322 | 5x10$^{+7}$ | +8 |
| Tetrachloroethylene | 127-18-4 | I | L | 3 | 5.1x10$^{-2}$ | 165.8 | 8x10$^{0}$ | +1 |
| Toxaphene | 8001-35-2 | I | S | 2B | 1.13 | 414 | 5x10$^{+2}$ | +3 |
| Trichloroethylene | 79-01-6 | I | L/S | 3/2B | 1.1x10$^{-2}$ | 131.4 | 1x10$^{0}$ | 0 |
| Vinyl chloride | 75-01-4 | S | S | 1 | 1.75x10$^{-2}$(I) | 62.5 | 1x10$^{0}$ | 0 |

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

[b] Animal slopes are 95% upper-bound slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear nonthreshold model. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available. The slope value is an upper bound in the sense that the true value (which is unknown) is not likely to exceed the upper bound and may be much lower, with a lower bound approaching zero. Thus, the use of the slope estimate in risk evaluations requires an appreciation for the implication of the upper bound concept as well as the "weight of evidence" for the likelihood that the substance is a human carcinogen.

[c] The potency index is a rounded-off slope in (mmol/kg/day)$^{-1}$ and is calculated by multiplying the slopes in (mg/kg/day)$^{-1}$ by the molecular weight of the compound.

the molecular weight of 97 gives a potency index of 1.1 x $10^{+2}$. Rounding off to the nearest order of magnitude gives a value of $10^{+2}$, which is the scale presented on the horizontal axis of Figure 10-7. The index of 1.1 x $10^{+2}$ lies in the third quartile of the 54 suspect carcinogens.

Ranking of the relative potency indices is subject to the uncertainty involved in comparing potency estimates for a number of chemicals based on different routes of exposure in different species using studies whose quality varies widely. Furthermore, all the indices are based on estimates of low-dose risk using linear extrapolation from the observational range. Thus, these indices are not valid for the comparison of potencies in the experimental or observational range if linearity does not exist there. Furthermore, for vinylidene chloride this quantitative estimate is subject to the further uncertainties of the limited evidence of carcinogenicity and the response in mice only at the higher dose.

### 10.5.6 Summary

10.5.6.1 QUALITATIVE -- Eighteen animal studies have been identified which provide information about the carcinogenic potential of VDC. Eleven of the studies involve inhalation exposure, five are oral (four gavage, one drinking water), and one each by skin application and subcutaneous injection. Some of the studies were designed and conducted as cancer bioassays while others were conducted to detect toxicity. None of the 11 inhalation studies had a full lifetime exposure, the exposures being for 12 months or less, while 3 of the 5 gavage studies were lifetime exposure.

The carcinogenicity of vinylidene chloride was evaluated by inhalation exposure of both sexes of Swiss mice and Sprague-Dawley rats to vinylidene chloride concentrations as high as those maximally tolerated (25 ppm for mice and 150 ppm for rats) for 12 months followed by lifetime observation. In addition, Chinese hamsters were exposed to 25 ppm vinylidene chloride (less than a maximally tolerated dose) for 12 months followed by lifetime observation (Maltoni et al., 1985). A statistically significant increase in kidney adenocarcinomas was found in male Swiss mice, and, although a statistically significant increase in mammary carcinomas in treated female Swiss mice was evident, the investigators concluded that a direct relationship between this response in female mice and vinylidene chloride treatment remains open, largely due to stated results of their statistical analysis, indicating a weaker response when the data are adjusted for survival and lack of a dose-related response over the course of the study. A carcinogenic effect of vinylidene chloride in Sprague-Dawley rats and Chinese hamsters was not apparent. The 12-month exposure period used in this study was below lifetime exposures.

Additional inhalation exposure studies of vinylidene chloride in animals have been done (Table 10-23). Exposure of male and female Sprague-Dawley rats to 25 ppm and 75 ppm vinylidene chloride for 18 months followed by 6 months of observation did not show a carcinogenic effect (McKenna et al., 1982); however, the exposure levels used did not appear to be overtly toxic, and use of additional higher exposure levels might have provided a broader evaluation for carcinogenicity.

No statistically significant increase in tumor formation in treated animals was shown in any of the following separate studies of exposure to vinylidene chloride:

(1) Male and female Wistar rats exposed to 200 ppm for 5 months and 100 ppm for 7 months followed by observation for an additional 12 months (Viola and Caputo, 1977);

(2) Male and female Sprague-Dawley rats exposed to 75 ppm and 100 ppm for an unspecified duration (Viola and Caputo, 1977);

(3) CD-1 mice and CD rats exposed to 55 ppm and observed for a period of 12 months (Lee et al., 1978);

(4) CD-1 mice exposed to 55 ppm for as long as 6 months and observed for 12 additional months (Hong et al., 1981); and

(5) CD rats exposed to 55 ppm for as long as 10 months and observed for an additional 12 months (Hong et al., 1981).

The known exposure periods of 12 months or less used in these studies were shorter than potential lifetime exposure periods, and use of more than one exposure level in the studies with CD-1 mice and CD rats could have provided a stronger evaluation of dose-response.

Several carcinogenicity studies in which vinylidene chloride was administered orally to experimental animals have been reported as negative. These studies include:

(1) Gavage administration of vinylidene chloride in olive oil at doses as high as 20 mg/kg to male and female Sprague-Dawley rats for 12 months, followed by lifetime observation (Maltoni et al., 1985);

(2) Administration of vinylidene chloride in drinking water at levels of maximum water solubility (200 ppm) to male and female Sprague-Dawley rats for 2 years (Quast et al., 1983; Humiston, et al., 1978);

(3) Gavage administration of vinylidene chloride in corn oil to male and female Fischer 344 rats at 1 and 5 mg/kg and male and female B6C3F1 mice at 2 and 10 mg/kg for 2 years (NCI/NTP, 1982);

(4) Gavage administration of 150 mg/kg of vinylidene chloride in corn oil to pregnant BDIV rats on day 17 of gestation, followed by weekly gavage doses of 50 mg/kg of vinylidene chloride to the pups for their lifetimes following birth (Ponomarkov and Tamatis, 1980).

The 12-month exposure in Sprague-Dawley rats given vinylidene chloride by gavage was below the potential lifetime exposure, and a maximally tolerated dose does not appear to have been selected. The lack of a clear effect on survival and body weight in Fischer 344 and B6C3F1 mice given vinylidene chloride in corn oil suggests that higher doses could have been tested to challenge the animals more strongly for carcinogenicity. A broader evaluation of vinylidene chloride carcinogenicity in BDIV rats might have been possible with the addition of more than the one dose level used in the study, and with more than one treatment each week.

Vinylidene chloride was not carcinogenic in female ICR/Ha mice when applied to the skin at 121 mg/mouse three times per week, or when injected subcutaneously at 2 mg/mouse once weekly at maximum tolerated doses in lifetime studies (Van Duuren et al., 1980). When a single application of 121 mg/mouse of vinylidene chloride was followed by repeated application of the tumor-promoting agent phorbol myristate acetate, skin papillomas resulted in ICR/Ha mice.

One epidemiologic study on a population on 138 workers (Ott et al., 1976) showed no carcinogenic effects attributable to vinylidene chloride. At the time the study was published in 1976, the dates of first exposures for 55 workers were between 1960 and 1969, thus indicating that nearly 40% of the workers had no more than 16 years latency since first exposure and that continued follow-up could strengthen the evaluation for carcinogenicity.

10.5.6.2 QUANTITATIVE -- Only the Swiss mice inhalation study of Maltoni et al. (1985) provides sufficient data for a quantitative cancer risk estimate for vinylidene chloride. This estimate is made under the presumption that vinylidene chloride is a human carcinogen. Based on kidney adenocarcinomas in male mice, the upper-bound incremental unit risk estimate for cancer from a lifetime of continous exposure to 1 ppb (vinylidene chloride) (air) is 2.0

$\times 10^{-4}$ [$5.0 \times 10^{-5} (\mu g/m^3)^{-1}$]. An estimate of the upper-bound incremental unit risk for 1 μg/L of vinylidene chloride in drinking water is $1.7 \times 10^{-5}$, based on the highest slope estimate from negative gavage and drinking water studies in rats and mice. The route of administration for these studies is more appropriate for extrapolating to drinking water exposures. The relative potency of vinylidene chloride, expressed in molar units, is $1.1 \times 10^{+2}$ $(mmol/kg/day)^{-1}$. Among 54 chemicals that the CAG has evaluated as suspect carcinogens, vinylidene chloride ranks in the third quartile.

### 10.5.7 Conclusions

Evidence of the carcinogenicity of vinylidene chloride was found in Swiss mice in an inhalation study (Maltoni et al., 1985). Although the exposure period was only 12 months, in which the mice were exposed for only 4 hours daily, kidney adenocarcinomas, a rare tumor type, were diagnosed in treated male Swiss mice. A statistically significant increase of mammary carcinomas was evident in female mice, and a statistically significant increase in pulmonary adenomas occurred in mice of both sexes at both dose levels. However, because no clear dose-response relationship exists, a direct correlation between induction of mammary carcinomas or pulmonary adenomas and vinylidene chloride exposure is uncertain, and therefore, the response is only weakly supportive in a weight-of-evidence context. Statistically significant increases in mammary tumors were observed in two studies in female Sprague-Dawley rats exposed to vinylidene chloride by inhalation and in drinking water, but these increases are not considered to be treatment-related since this strain of rats has a high background incidence of mammary tumors and there was no dose-reponse relationship between tumor incidence and exposure to vinylidene chloride. Van Duuren et al. (1979) demonstrated that vinylidene chloride acts as a tumor initiator in mouse skin.

Although no other inhalation studies on the carcinogenicity of vinylidene chloride demonstrated any statistically significant increase of tumors, all of these studies had one or more deficiencies: shortened exposure or observation periods, did not reach a maximum tolerable dose, and/or used only a single dose level. None of the five oral studies show a statistically significant response, yet only two of the five were lifetime, multiple dose studies and the dosing appears to be less than the MTD.

There is no adequate epidemiologic data to assess the carcinogenicity of vinylidene chloride in humans.

Vinylidene chloride has been shown to be mutagenic in several strains of bacteria, in yeast, and in the plant *Tradescantia*; it has even been used as a positive control in a bacterial study assessing the mutagenic potential of volatile compounds. Vinylidene chloride does require metabolic activation in order to produce a mutagenic response in bacteria and yeast; livers from a variety of species, including man, have been used to metabolically activate vinylidene chloride in *in vitro* assays.

Vinylidene chloride can be metabolized to intermediates capable of reacting with macromolecules. At doses similar to those used in the positive mouse bioassay, covalent binding of active metabolites to macromolecules is greater in the kidney (the target organ in the bioassay) than in the liver; DNA alkylation does occur at low levels and is also greater in the kidney than in the liver, particularly in the mouse.

Applying the International Agency for Research on Cancer (IARC) approach for classifying carcinogenic agents, the level of evidence for vinylidene chloride carcinogenicity in animals is considered to be limited. Applying the IARC approach for evaluating epidemiologic data, the currently available data is considered inadequate for making a judgment on the carcinogenicity of vinylidene chloride. Based on the overall evidence, vinylidene chloride would be a Group 3 chemical according to the IARC approach, and by definition, a Group 3 chemical cannot be classified as to its carcinogenic potential for humans. However, the mutagenic activity of vinylidene chloride, its chemical structure, its activity as a tumor initiator in mouse skin, and the ability of metabolites to react with DNA, raise a concern that the total body of evidence should not be lightly dismissed. The negative results from many studies do not contradict this position since unfortunately all of the studies are flawed in their sensitivity to detect a carcinogenic response.

Based on EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1984), the animal evidence for vinylidene chloride would result in an overall classification of Group C, meaning that the chemical is a "possible" human carcinogen.

Although vinylidene chloride has only limited animal evidence for carcinogenicity (Group 3 chemical according to the IARC classification system and Group C according to the EPA classification system), the kidney adenocarcinomas in male Swiss mice provide data that have been used for estimating an upper bound of the incremental unit risk, under the presumption that vinylidene chloride is a potential human carcinogen. The upper-limit incremental cancer risk is $5.0 \times 10^{-5}$ for a lifetime continuous exposure to 1 $\mu$g (vinylidene chloride)/$m^3$ in air and $1.7 \times 10^{-5}$ for exposure to 1 $\mu$g/L in drinking water. Expressed in terms of 1 $\mu$g/kg bw/day continuous lifetime exposure to vinylidene chloride, the upper-limit incremental cancer risk is $1.16 \times 10^{-3}$. Expressed in terms of relative potency, vinylidene chloride would rank in the third quartile among the 54 chemicals that the CAG has evaluated as suspect or known human carcinogens.

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